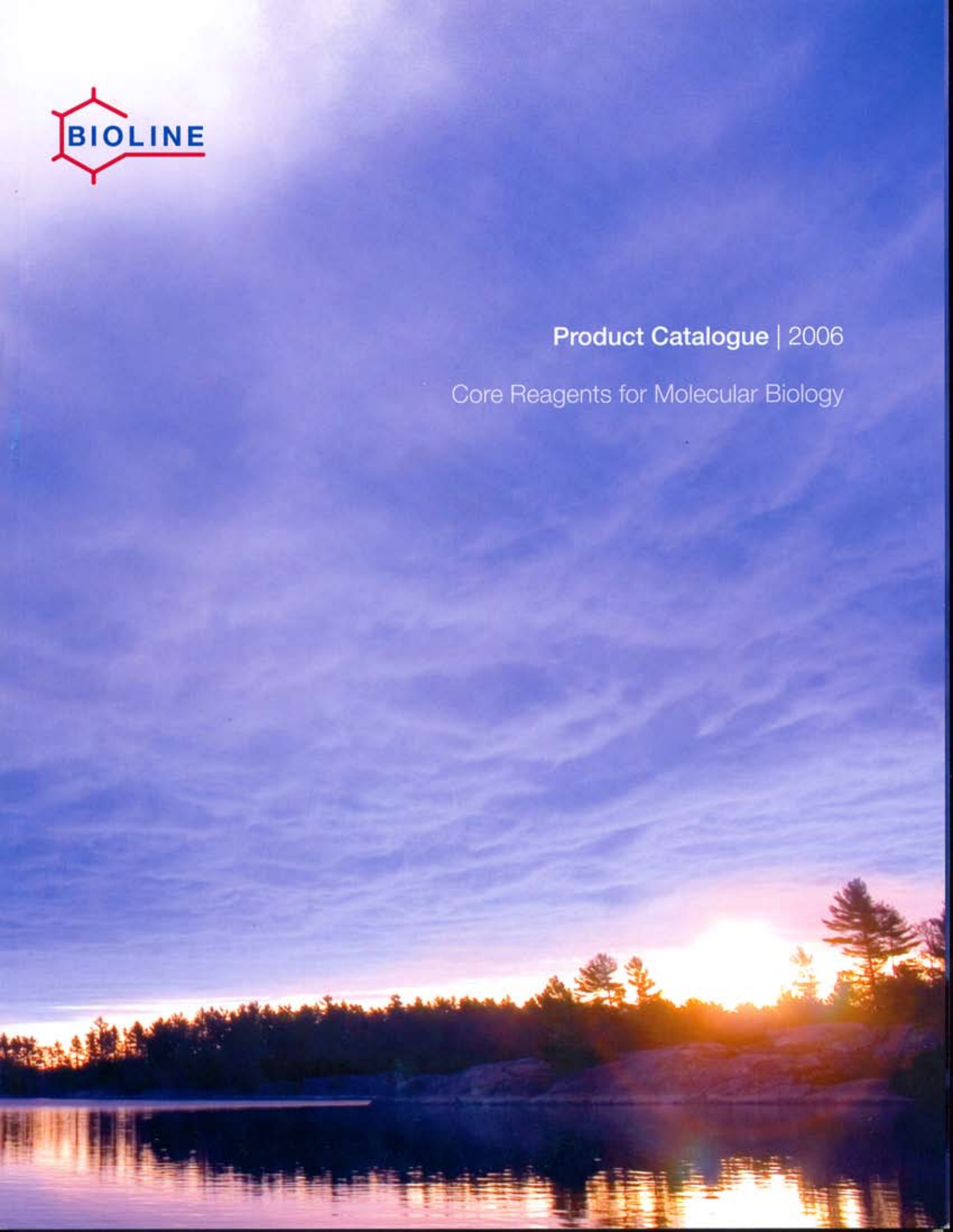
BIOLINE

Product Catalogue | 2006

Core Reagents for Molecular Biology



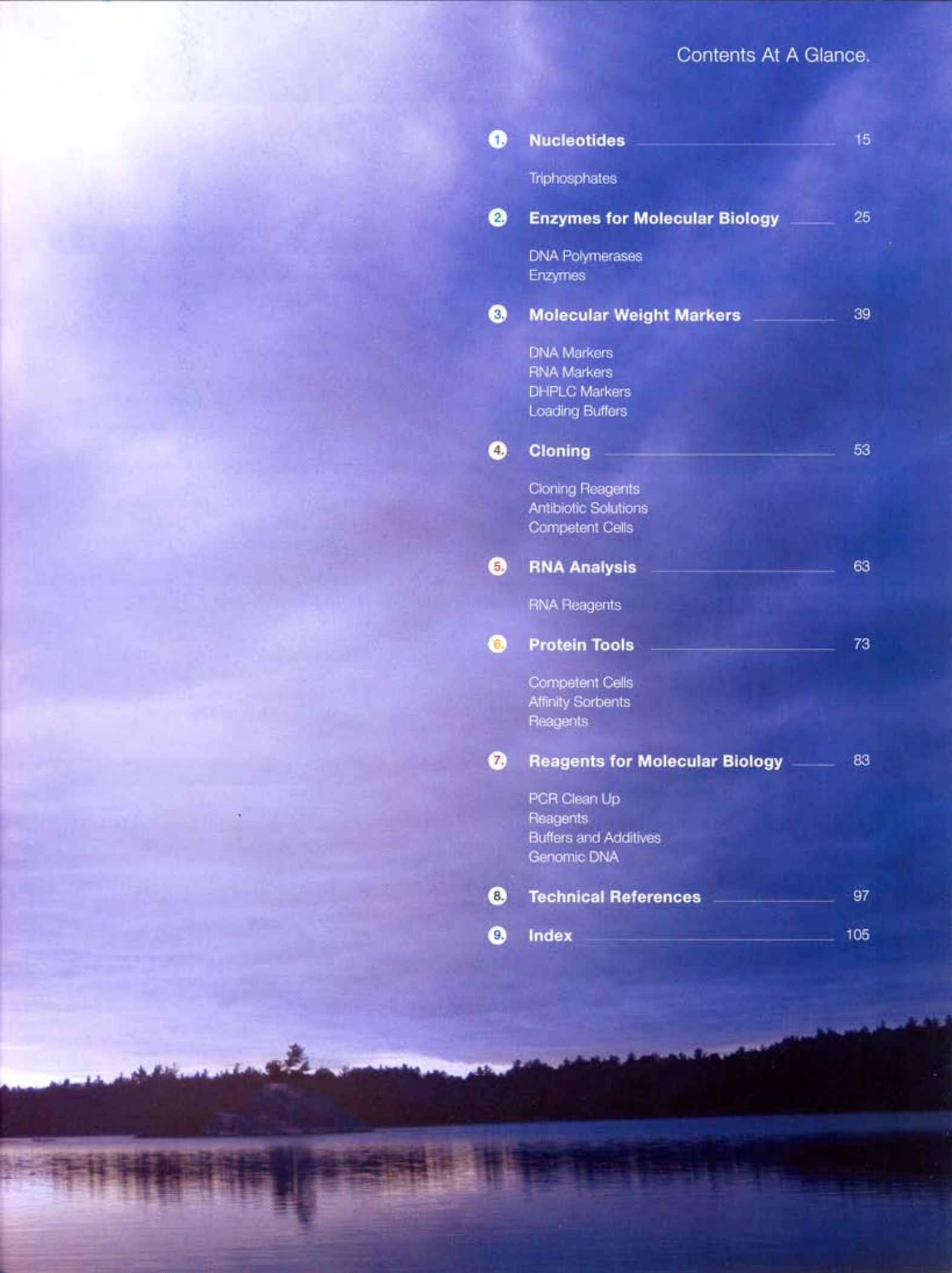
Welcome to your new Bioline catalogue for 2006. At Bioline, we strive to deliver top-grade reagents coupled with excellent customer service. In this catalogue, we deliver several new molecular biology reagents, which are the result of our ongoing investment into product development. We also collaborate with laboratories in many nations in order to identify innovative ideas that could develop into mainstream reagents through licensing programs. If you believe your laboratory has an innovative development, we would be delighted to hear from you.

Bioline has long been established as one of the world's few primary manufacturers of extremely pure dNTPs. As the requirement for ultra-pure dNTPs constantly grows for more demanding assays, such as real-time PCR, scientists from around the world have come to rely on Bioline to deliver top-grade dNTPs.

We look forward to being of service in your laboratory and welcome any suggestions you may have.

Lake Ontario, Canada

Contents At A Glance.



General Information

Table of Contents.

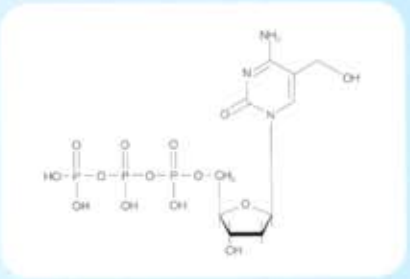
Table of Contents

Welcome to your new Bioline catalogue for 2006.
We are pleased to introduce many new reagents this year.

1. Nucleotides

Hydroxymethylated dCTP 20

Bioline have developed a novel method for producing highly purified hydroxymethylated dCTP (HMdCTP), which can be used in a number of molecular biological applications and offers significant advantages over normal dCTP. Bioline is the first company to provide scientists with an opportunity to synthesise uniformly hydroxymethylated DNA.



2. Enzymes for Molecular Biology

Mango*Taq* DNA Polymerase 36

A new formulation of *Taq* DNA polymerase supplied with a reaction buffer containing two dyes that separate during electrophoresis, providing quick reference points for monitoring gel migration.

TBE Buffer
(5, 10, 15 and 20 µl)

Red and orange dyes after electrophoresis. Differing volumes of the amplification reactions subjected to electrophoresis.

3. Molecular Weight Markers

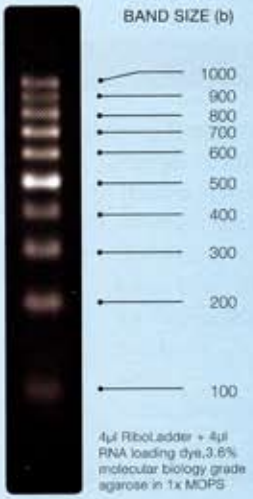
RiboLadder Short 47

A single-stranded RNA molecular weight marker with band sizes ranging from 100b - 1000b.

RiboLadder Long 47

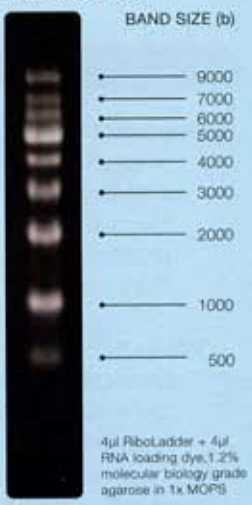
A single-stranded RNA molecular weight marker with band sizes ranging from 500b - 9000b.

RiboLadder Short



4µl RiboLadder + 4µl RNA loading dye,3.6% molecular biology grade agarose in 1x MOPS

RiboLadder Long

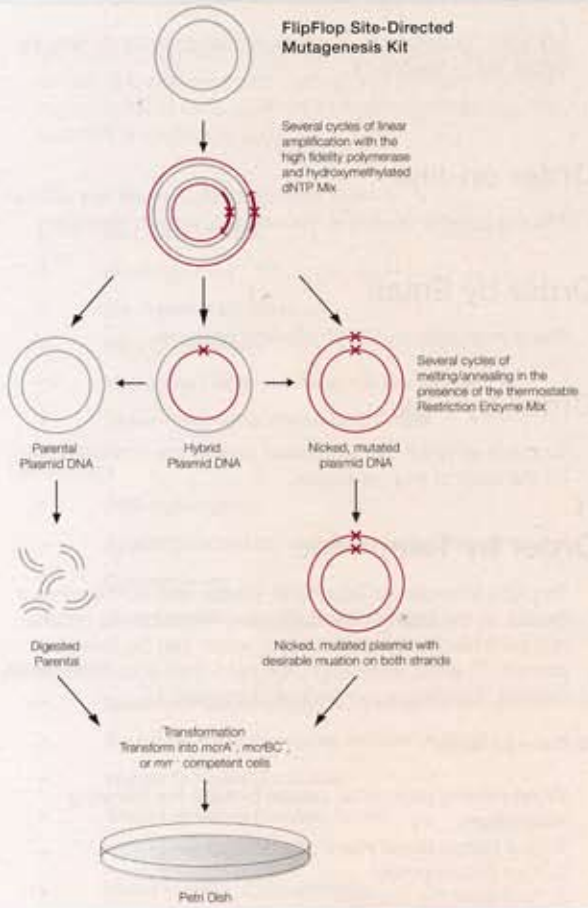


4µl RiboLadder + 4µl RNA loading dye,1.2% molecular biology grade agarose in 1x MOPS

4. Cloning

FlipFlop Site-Directed Mutagenesis Kit 54

The FlipFlop Site-Directed Mutagenesis Kit is a newly developed, highly efficient and fast working system for the generation of oligonucleotide-directed mutations and is designed to carry out site-directed mutagenesis in virtually any double-stranded plasmid of up to 12Kb. The Kit does not require any special treatment of the template, the entire mutagenesis reaction is completed within 15 thermal cycles and the protocol is simple and easy to use. Once mutagenesis is complete, the mutagenised plasmids are ready for transformation.



Competent Cells 59-61

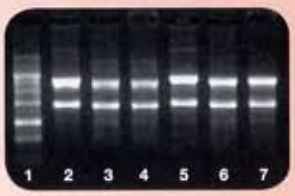
Bioline offers a wide range of *E.coli* host strains to facilitate High Efficiency Transformation and Protein Expression. Bioline maintains rigorous quality control standards to ensure lot-to-lot consistency and the highest transformation efficiencies possible. Several cloning strains are available which include desirable genetic markers, high transformation efficiencies, and convenient packaging. In addition, Bioline offers a series of BL21 Competent Cells for optimal protein expression and expression control.

α-Select Competent Cells
ElectroSHOX
BIO*Blue*
BL 21 Competent Cells
CH3-Blue

5. RNA Analysis

TRIsure 69

TRIsure is a ready-to-use reagent for the isolation of total RNA from cells and tissues. TRIsure maintains the integrity of the extracted RNA, while disrupting cells and subsequently dissolving cell components. The isolated RNA is suitable for any downstream application such as RT-PCR, hybridisation assays, or *in vitro* transcription.



RNA extracted from 3T6 cells and mouse tissue, using Bioline TRIsure and Competitor I.

Lane 1: RiboLadder Long
Lane 2: 4µg of total RNA from 3T6 cells - Competitor I
Lane 3: 4µg of total RNA from mouse kidney tissue - Competitor I
Lane 4: 4µg of total RNA from mouse liver tissue - Competitor I
Lane 5: 4µg of total RNA from 3T6 cells - Bioline TRIsure
Lane 6: 4µg of total RNA from mouse kidney tissue - Bioline TRIsure
Lane 7: 4µg of total RNA from mouse liver tissue - Bioline TRIsure

6. Protein Tools

Affinity Sorbents 78-80

The new range of Bioline celluloses enable labs to perform one-step purifications from crude lysate to >95% pure protein, with high binding affinity and capacity. At present three celluloses are made available in two different pack sizes.

Metal Chelating Cellulose
Glutathione Cellulose
Heparin Cellulose

Ordering Information

For your convenience, you may choose to order on-line, by Email, fax or telephone.

Order on-line

Place you order on-line at our website: www.bioline.com

Order by Email

Place your order by Email: info@bioline.com

Order by Fax

To place an order by Fax, please refer to the contact details on the back of this catalogue.

Order by Telephone

To place an order by Telephone, please refer to the contact details on the back of this catalogue. All telephone orders require a hard copy confirmation, which can be faxed or posted. To avoid duplication, the hard copy should be clearly marked "Confirmation – Do Not Duplicate".

To Place an Order

When placing your order, please provide the following information:

1. Your name, department and telephone number
2. Your order number
3. Your shipping address
4. Your billing address and telephone number
5. Catalogue number, product description and quantity
6. Any discount codes
7. VAT status and exemption certificate as required (UK only)

A complete list of international distributors can be found in the following pages.

General Enquiries

Customers are invited to make any enquiries to info@bioline.com

Conditions of Use

Products are sold for research and laboratory use only. Our products are NOT to be administered to humans or used for medical diagnosis.

Disclaimer

Bioline shall not be responsible for injury or damages resulting from the use or misuse of any of its products.

Payment Terms

Terms are net 30 days. Prices are subject to change without notice.

Credit Cards

In order to facilitate purchases for our customers, we accept payment by credit card in the United Kingdom and U.S.A.

Shipping & Delivery

Depending upon the nature of the product, shipments are made by post, on blue ice-bricks or on dry-ice.

Orders received by 3.00pm (UK, Australia and Germany) or 4.00pm (USA) will generally be shipped the same day for next day delivery.

Shipping days are Monday through Thursday and the following shipping and handling charges are added to the invoice: UK £13.50, USA $24, Australia $20 and Germany €12.

Conditions of Sale

Bioline does not agree to and is not bound by any other terms or conditions, unless expressly agreed to in writing by Bioline. In the absence of any formal agreement to the contrary Bioline reserves the right to change any terms or conditions without notice.
Please refer to the Terms and Conditions of Sale for more information.

Returns

Owing to the temperature requirements of our products, it is not practical to resell returned goods, as we cannot be assured of their quality. Therefore, we regret that in the event of a purchasing error it is our policy not to accept returned goods.

Free Sample Policy

Existing customers will be aware of our policy on samples. Most of the products in our range are readily available in sample sizes at no cost, enabling a potential user to measure product performance before making a decision to purchase.

Requests for Literature

Please request literature online at www.bioline.com or by sending an Email to info@bioline.com

Bulk and Custom Program

Bioline has expanded its Bulk Program so that we can offer laboratories:

- **Custom bulk configurations**
- **Custom bulk packaging**
- **Private label OEM manufacturing**
- **Scheduled shipments**

Technical Support

Bioline has a fully trained customer service and technical support team skilled in the use of Bioline products and all related applications. For prompt and accurate technical support please contact us at any of the following:

Email
tech@bioline.com

UK
Tel: 0044 (0)20 8830 5300
Fax: 0044 (0)20 8452 2822

USA
Tel: 781 830 0360
Fax: 781 830 0205

Germany
Tel: 0049 (0)3371 681229
Fax: 0049 (0)3371 681 244

Australia
Tel: 0061 (0)2 9209 4180
Fax: 0061 (0)2 9209 4763

Web Tools

We have added new interactive web tools to our website to help you with your calculations and conversions. The following list of tools, calculators and converters can be found at www.bioline.com

Nucleic and Amino Acids Sequence Tools

- **Sequence Utilities**
- **Reading Aloud**
- **Six-Frame Translation**
- **Rare Codon Search**
- **Mixed and Random Nucleotide Sequences**
- **Three-/ one-letter Amino Acid Codes**

Calculators

- **PCR Optimisation**
- **Spectrophotometric Measurement of Nucleic Acid**
- **Concentration**
- **Time Estimation for Bacterial Growth**

Converters

- **Speed of Rotation [krpm] to Relative Centrifuge Force [kg]**
- **Radioactivity to Biological Amount of dNTPs**
- **Weight to Moles (Proteins)**
- **Weight to Moles (Nucleic Acids)**
- **DNA to Protein**
- **Moles to Molar Concentration**
- **Conversion of Units of Measurement**

For Converters, Sequencing Tools & Calculators visit www.bioline.com

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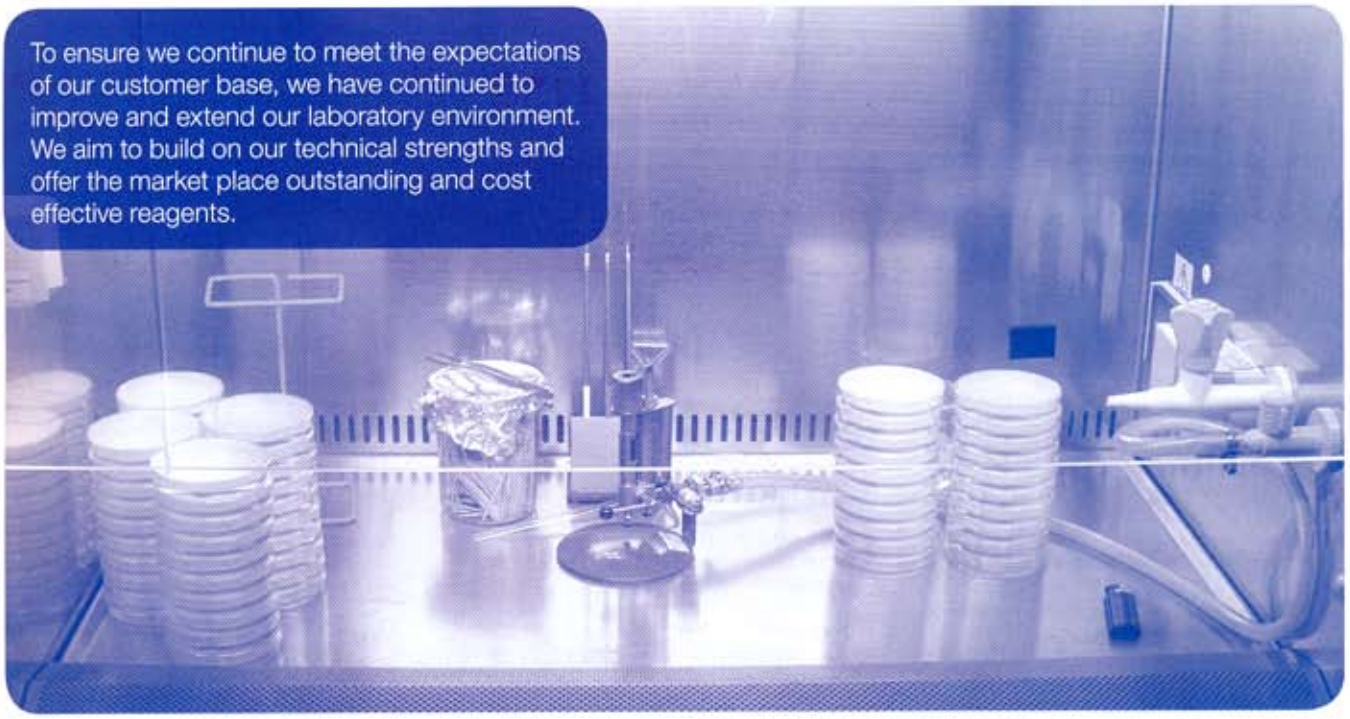


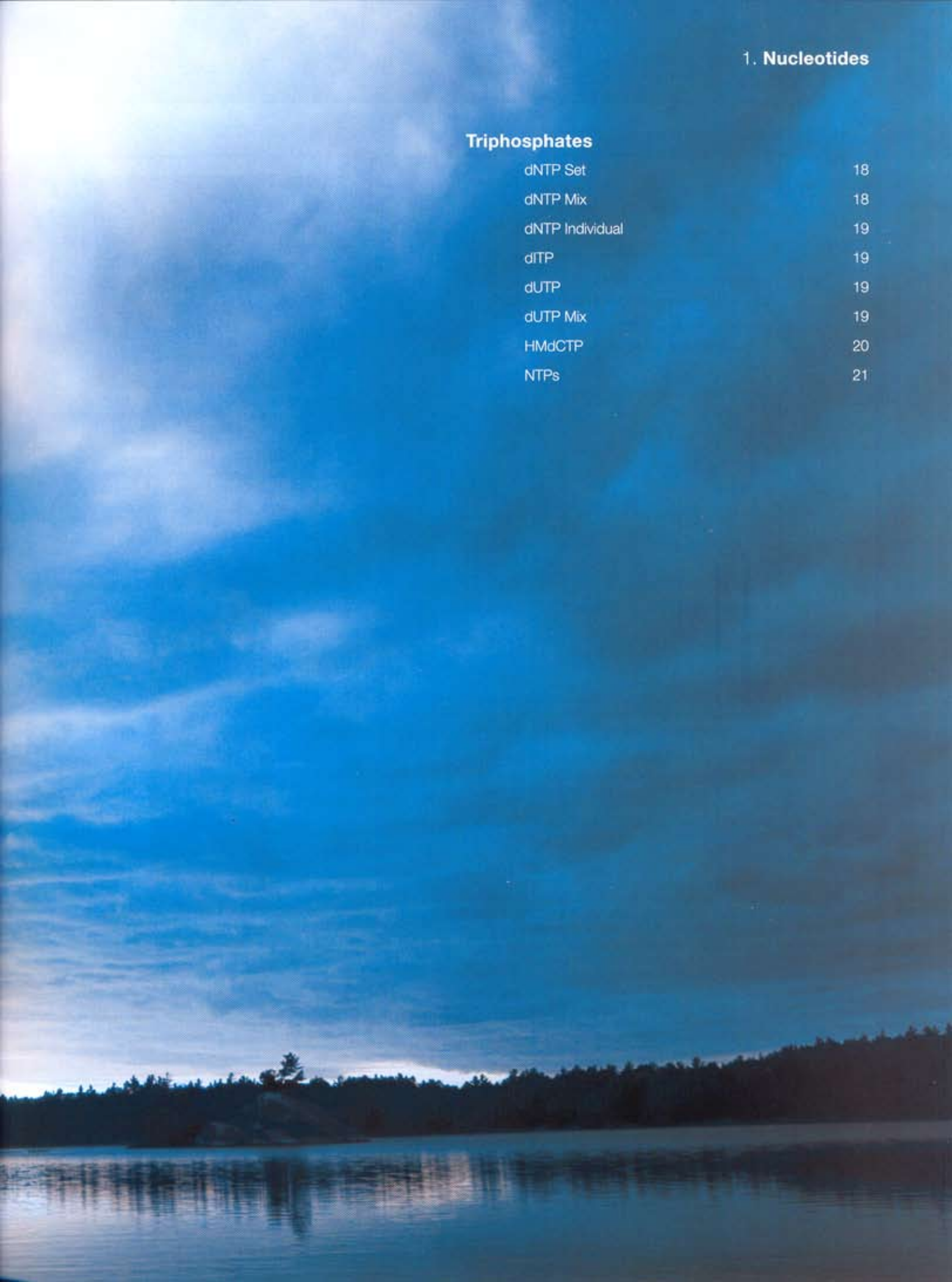
To ensure we continue to meet the expectations of our customer base, we have continued to improve and extend our laboratory environment. We aim to build on our technical strengths and offer the market place outstanding and cost effective reagents.

Bioline participates in strategic alliances with renowned academic laboratories.

The University Of Sheffield.

UCL

Triphosphates



Ultra-Pure dNTPs

Features

- dNTPs direct from the primary manufacturer
- Extremely high purity
- Functionally tested in a wide range of assays to guarantee outstanding results
- Enzymatic synthesis of dNTPs, to eliminate PCR inhibitors
- Strict quality control measures and performance tests on each batch
- Newly developed hydroxymethylated dCTP
- Lithium Salts formulation for improved dNTP stability and extended shelf life
- Available in sets or mixes in varying volumes, from 1ml to multi-litre bulk packages

Facility

Bioline has a purpose-built facility in Germany producing ultra-pure deoxynucleotide triphosphates (dNTPs) on an industrial scale, and supplies ultra-pure dNTPs to laboratories all over the world.

Performance and Sensitivity

Purified dNTPs are recommended for use in highly sensitive techniques such as long-distance PCR, RT-PCR, multiplex, Real-Time applications, mutagenesis, and low-copy amplifications (Fig 1.1), Bioline ultra-pure dNTPs meet exacting standards and can be employed in the most sensitive assays, such as low-copy or rare message assays, to achieve successful PCR and outstanding results.

Purity

Ultra-pure dNTPs are enzymatically synthesised from deoxynucleotide monophosphates (dNMPs) by a process of enzymatic phosphorylation. This process uses highly specific enzymatic systems, eliminating impurities and PCR inhibitors such as modified nucleotides and pyrophosphates. The dNTPs are purified with preparative HPLC and possess at least 99% purity.

Quality Control

The dNTPs are tested for the absence of DNase, RNase, Protease and nicking activity. Tests to confirm the absence of human and bacterial DNA are also carried out. Each batch is validated rigorously for purity by HPLC and tested for performance in a wide range of PCR templates, including a long-distance 20Kb functional test (Fig 1.2).

DHPLC

DNA fragments amplified using Bioline dNTPs have been assayed for use on DHPLC. dNTP purity is a critical consideration for DHPLC usage, as there must be no contamination by monophosphates or incorrectly synthesised/modified bases. Contaminating nucleotides or derivatives may cause premature strand termination and result in unwanted peaks (Fig 1.3).

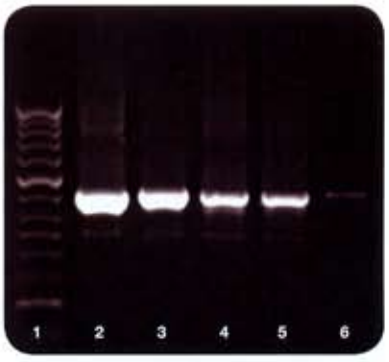


Fig 1.1 Low Copy Assay
A fragment of the ß-actin gene of human genomic DNA was amplified using Bioline dNTPs in a 50µl reaction volume, with different template concentrations.

Lane 1: HyperLadder II
Lane 2: 50ng of genomic DNA
Lane 3: 5ng of genomic DNA
Lane 4: 2.5ng of genomic DNA
Lane 5: 0.5ng of genomic DNA
Lane 6: 0.1ng of genomic DNA

Fig 1.2 High dNTP Purity for Long Distance PCR
Lane 1: HyperLadder I (top band = 10 Kb)
Lane 2: Amplification of a 15 Kb DNA fragment
Lane 3: Amplification of a 20 Kb DNA fragment

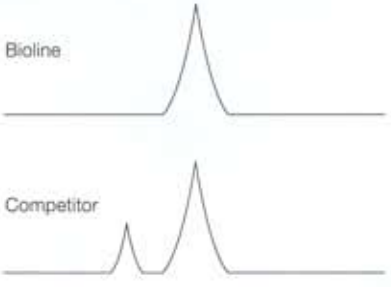
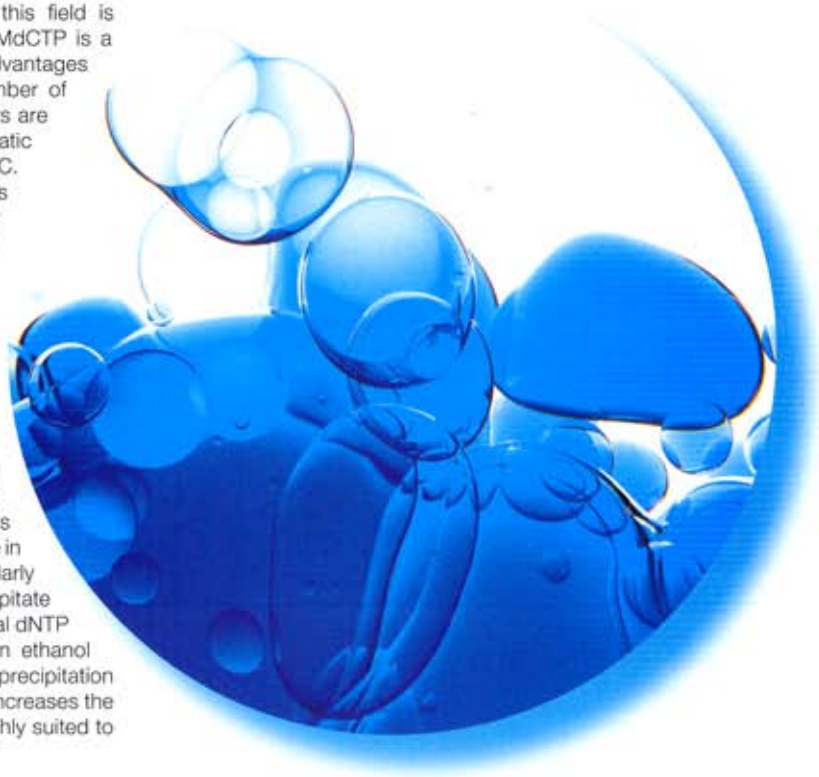


Fig 1.3 DHPLC trace illustrating dual products formed as a result of premature strand termination in competitor dNTP.

New Development in Nucleotides Area

Bioline is constantly developing and enhancing its production capacity and expertise in the nucleotide area. The most recent development from our laboratories in this field is hydroxymethylated dCTP (HMdCTP). Bioline HMdCTP is a novel nucleotide analogue offering significant advantages over normal dCTP, and can be used in a number of molecular biology applications. Bioline HMdCTPs are manufactured using a unique method of enzymatic synthesis and possess at least 99% purity by HPLC. HMdCTPs can be used to generate PCR products in which cytosines are uniformly replaced by hydroxymethylated cytosines and have exciting applications in forensic DNA analysis, site-directed mutagenesis and the production of DNA fragments resistant to cleavage.



Extended Storage Life

dNTPs are normally supplied dissolved in salts of either Lithium or Sodium. Bioline dNTPs are presented in Lithium salts, which are more resistant to repeated freeze/thaw cycles and remain sterile over the entire storage period (lithium ions exhibit bacteriostatic activity towards various microorganisms). dNTPs are more soluble in Lithium salts than in Sodium salts. This is particularly important for dGTP which has a tendency to precipitate during freezing and cause an imbalance in the final dNTP concentration. Lithium salts are more soluble in ethanol than Sodium salts, so their removal by ethanol precipitation is more efficient, as it reduces salt artefacts and increases the legibility of sequencing gels. Lithium salts are highly suited to PCR, sequencing and labelling applications.

Bioline dNTPs are stable for 12 months when stored in a -20°C constant-temperature freezer.

Note: As significant hydrolysis occurs when dNTPs are stored at low concentrations, always store nucleotide stock solutions at a concentration of ≥10mM.

Configuration

Bioline dNTPs are available as both convenient 100mM sets in three pack sizes, and as ready-to-use dNTP mixes that can be added directly to amplification reactions, and are designed to save time, reduce the risk of contamination and ensure the reproducibility of results. The dNTP solutions are ready-to-use at pH 7.5 in Lithium salts, which offer improved stability of the dNTPs in reactions, and a longer shelf life as compared with Sodium salts.

The following descriptions are intended to ensure that customers are fully aware of what is on offer, indicating the three parameters of interest: Pack Size (concentration of dNTP), Final Concentration (absolute amount of dNTP supplied) and Presentation (final volume supplied).

Free Samples

Samples of our dNTP range of products are readily available in sample sizes at no cost. If your laboratory is not already using Bioline dNTPs, please contact Bioline for a free sample.

Bulk Orders

Bioline is a primary manufacturer and can accommodate multi-litre orders for dNTPs. Please contact your nearest Bioline representative or distributor for a quote.

dNTP Set

PACK SIZE	FINAL CONC.	PRESENTATION	CAT NO.
4 x 25µmol	100mM total	4 x 250µl	BIO-39025
4 x 100µmol	100mM total	4 x 4 x 250µl	BIO-39026
4 x 500µmol	100mM total	4 x 20 x 250µl	BIO-39027

Features

- Ultra-pure: >99% triphosphate by HPLC
- Enhanced storage life
- Convenient solutions at pH 7.5

Applications

- Standard and Long PCR
- Real-Time PCR
- Reverse transcription
- Mutagenesis
- DNA labelling and sequencing

Description

A set of ready-to-use molecular grade dNTP solutions supplied as sets: dATP, dCTP, dGTP and dTTP, for use in DNA polymerisation reactions, DNA labelling and sequencing processes. Dependable PCR grade.

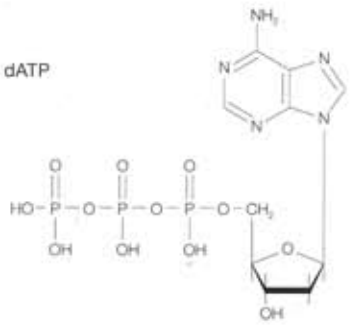
Storage Conditions

dNTP Set can be stored for 12 months at -20°C. Avoid multiple freeze/thaw cycles. For long-term usage, aliquoting is recommended.

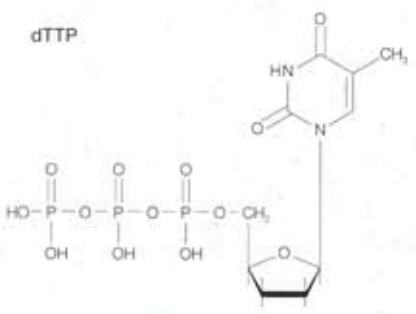
Associated Products

PRODUCT	CAT NO.	CHAPTER
BIOTAQ	BIO-21040	Enzymes for Molecular Biology
IMMOLASE	BIO-21046	Enzymes for Molecular Biology
Agarose Tablets	BIO-41028	Reagents for Molecular Biology
HyperLadder I	BIO-33025	Molecular Weight Markers

dATP



dTTP



dNTP Mix

PACK SIZE	FINAL CONC.	PRESENTATION	CAT NO.
10µmol	10mM total	1ml	BIO-39044
20µmol	40mM total	1 x 500µl	BIO-39043
50µmol	100mM total	1 x 500µl	BIO-39028
200µmol	100mM total	4 x 500µl	BIO-39029
100µmol	10mM total	10 x 1ml	BIO-39053

Features

- Ultra-pure: >99% triphosphate by HPLC
- Enhanced storage life
- Convenient solutions at pH 7.5

Applications

- Standard and Long PCR
- Real-Time PCR
- Dideoxy sequencing
- Mutagenesis
- cDNA sequencing

Description

A ready-to-use molecular grade dNTP Mix containing dATP, dCTP, dGTP and dTTP. The mix is designed to save hands-on time for researchers and minimise the possibility of contamination. Dependable PCR grade.

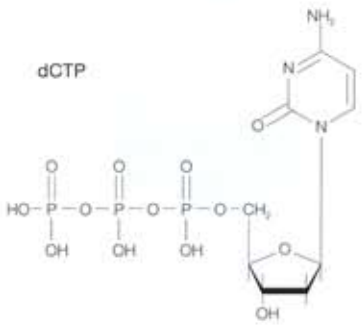
Storage Conditions

dNTP Mix can be stored for 12 months at -20°C. Avoid multiple freeze/thaw cycles. For long-term usage, aliquoting is recommended.

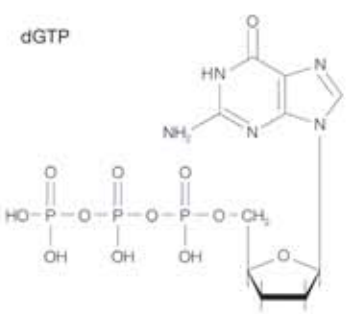
Associated Products

PRODUCT	CAT NO.	CHAPTER
BIOTAQ	BIO-21040	Enzymes for Molecular Biology
HyperLadder I	BIO-33025	Molecular Weight Markers
Agarose Tablets	BIO-41028	Reagents for Molecular Biology
Agarose	BIO-41026	Reagents for Molecular Biology

dCTP



dGTP



dNTPs Individual

PRODUCT	PACK SIZE	FINAL CONC.	PRESENTATION	CAT NO.
dATP	25µmol	100mM	1 x 250µl	BIO-39036
dCTP	25µmol	100mM	1 x 250µl	BIO-39038
dGTP	25µmol	100mM	1 x 250µl	BIO-39037
dTTP	25µmol	100mM	1 x 250µl	BIO-39039
dUTP	25µmol	100mM	1 x 250µl	BIO-39035
dITP	25µmol	100mM	1 x 250µl	BIO-39032

Features

- Ultra-pure: >99% triphosphate by HPLC
- Enhanced storage life
- Convenient solutions at pH 7.5

Applications

- Standard and Long PCR
- Real-Time PCR
- Dideoxy sequencing
- Mutagenesis
- cDNA sequencing

Description

Ready-to-use molecular grade individual dNTP solutions for use in DNA polymerisation reactions, DNA labelling, and sequencing processes.

Storage Conditions

dNTPs can be stored for 12 months at -20°C. Avoid multiple freeze/thaw cycles. For long-term usage, aliquoting is recommended.

Associated Products

PRODUCT	CAT NO.	CHAPTER
HyperLadder I	BIO-33025	Molecular Weight Markers
Agarose Tablets	BIO-41028	Reagents for Molecular Biology
Agarose	BIO-41026	Reagents for Molecular Biology

dUTP

PACK SIZE	FINAL CONC.	PRESENTATION	CAT NO.
25µmol	100mM	250µl	BIO-39035

Features

- High purity: >99% by HPLC
- Convenient solution at pH 7.5

Applications

- PCR assays
- Applications in which DNA substrates containing Uracil are required

Description

Ready-to-use molecular grade dUTP solution.

Storage Conditions

dUTP solution can be stored for 12 months at -20°C. Avoid multiple freeze/thaw cycles. For long-term usage, aliquoting is recommended.

Associated Products

PRODUCT	CAT NO.	CHAPTER
HyperLadder I	BIO-33025	Molecular Weight Markers
Agarose Tablets	BIO-41028	Reagents for Molecular Biology
Agarose	BIO-41026	Reagents for Molecular Biology

dUTP Mix (10mM each dATP, dCTP, dGTP, 20mM dUTP)

PACK SIZE	FINAL CONC.	PRESENTATION	CAT NO.
25µmol	50mM total	1 x 500µl	BIO-39041

Features

- PCR grade
- Convenient, pre-optimised, pre-mixed

Applications

- PCR assays

Description

dUTP Mix is a 50x solution which contains 10mM each of dATP, dCTP, dGTP and 20mM dUTP.

Storage Conditions

dUTP Mix can be stored for 12 months at -20°C. Avoid multiple freeze/thaw cycles. For long-term usage, aliquoting is recommended.

Associated Products

PRODUCT	CAT NO.	CHAPTER
HyperLadder I	BIO-33025	Molecular Weight Markers
Agarose Tablets	BIO-41028	Reagents for Molecular Biology
Agarose	BIO-41026	Reagents for Molecular Biology

dITP

PACK SIZE	FINAL CONC.	PRESENTATION	CAT NO.
25µmol	100mM	250µl	BIO-39032

Features

- High purity: >99% by HPLC
- Convenient solution at pH 7.5

Applications

- PCR assays
- Random mutagenesis
- DNA sequencing

Description

Ready-to-use molecular grade dITP solution.

Storage Conditions

dITP solution can be stored for 12 months at -20°C. Avoid multiple freeze/thaw cycles. For long-term usage, aliquoting is recommended.

Associated Products

PRODUCT	CAT NO.	CHAPTER
HyperLadder I	BIO-33025	Molecular Weight Markers
Agarose Tablets	BIO-41028	Reagents for Molecular Biology
Agarose	BIO-41026	Reagents for Molecular Biology

Hydroxymethylated dCTP

PACK SIZE	FINAL CONC.	PRESENTATION	CAT NO.
25µmol	100mM	1 x 250µl	BIO-39046

Features

- Ultra-pure: >99% triphosphate by HPLC
- Readily incorporated by standard DNA polymerases e.g. *Taq* family, Pfu, Tli, Tth, Bst, MMLV and AMV reverse transcriptases
- Available exclusively from Bioline
- Hydroxymethylated substrates ligated by standard ligases
- Convenient solution at pH 7.5

Applications

- Site-Directed Mutagenesis
- Substitution of dCTP in a wide variety of molecular biology assays
- Discrimination between the different DNA molecules synthesised in one or several PCR
- Structural and activity studies of the restriction/modification systems of different organisms
- Labelling of DNA *in vitro*
- Methylation studies
- Studies of Hydroxymethylated DNA/protein interaction

Introduction

5 Hydroxymethyl 2′ deoxycytidine 5′ triphosphate (HMdCTP) is a nucleotide existing in nature as a component of the DNA of T-even phages. The T-even DNA synthesising machinery is one of the most precise known in nature. T-even phages protect their own DNA from self-degradation, while infecting bacterial cells and degrading the host genome, by the phenomenon of two-step genome modification. In the first step, all phage cytosines are replaced by 5 hydroxymethylcytosine. This reaction involves the conversion of dCMP to HMdCMP catalysed by the T4 encoded enzyme, deoxycytidylate hydroxymethyltransferase.

In wild-type *E. coli*, mechanisms exist for discriminating between hydroxymethylated and non-hydroxymethylated DNA, such as the *mcr*BC system, which has not been fully understood until now. There is available a wide range of bacterial strains with deletions of these systems (e.g. Bioline CH3-Blue Chemically Competent Cells, Cat No. BIO-85040), which are able to make stable replications of hydroxymethylated DNA.

To date all restriction/modification systems have been investigated with natural substrates such as hydroxymethylated DNA isolated from T-even phages. Bioline is the first company to provide scientists with an opportunity to synthesise uniformly hydroxymethylated DNA for use in a wide variety of applications.

Description

Bioline have developed a novel method for producing highly purified HMdCTP. Using a unique enzymatic synthesis method, Bioline have been able to mimic the biological steps in the synthesis of HMdCTP from T-even phages. Highly purified HMdCTP can be used in a number of molecular biological applications and offers significant advantages over normal dCTP.

HMdCTP can be used as a substrate for several DNA polymerases under conditions that permit the amplification of DNA containing hydroxymethylated cytosine in place of cytosine (see Chapter 2 for a full listing of Bioline DNA polymerases). HMdCTPs can be used to discriminate between the different DNA molecules synthesised in one or several PCR cycles. By the use of appropriate enzymes, it is possible to separate the un-hydroxymethylated starting material from the hemi-hydroxymethylated intermediate (produced by a single primer extension reaction) and from the fully-hydroxymethylated end product.

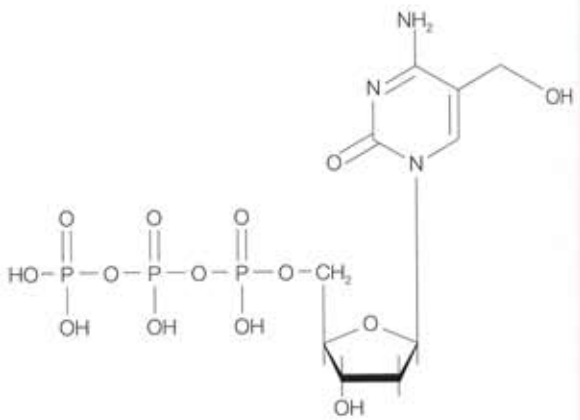
This ability to generate PCR products in which cytosine is uniformly replaced by hydroxymethylated cytosine can be applied to: (a) forensic DNA analysis, (b) the development of novel strategies for site-directed mutagenesis and (c) the production of DNA fragments resistant to cleavage by a wide range of restriction endonucleases, useful in the generation of cDNA libraries.

Storage Conditions

HMdCTP can be stored for 12 months at -20°C. Avoid multiple freeze/thaw cycles. For long-term usage, aliquoting

Associated Products

PRODUCT	CAT NO.	CHAPTER
FlipFlop Site-Directed Mutagenesis Kit	BIO-86030	Cloning
ACCUZYME	BIO-21052	Enzymes for Molecular Biology
AccuSure	BIO-21069	Enzymes for Molecular Biology
BIOTAQ	BIO-21060	Enzymes for Molecular Biology
CH3-Blue	BIO-85040	Cloning



NTPs

PRODUCT	PACK SIZE	FINAL CONC.	PRESENTATION	CAT NO.
NTP Set	4 x 25µmol	100mM	4 x 250µl	BIO-39052
NTP Mix	100µmol	100mM	1ml	BIO-39050

Features

- Suitable for *in vitro* transcription
- >98% pure by HPLC
- Supplied as 100mM solutions
- Sets or a convenient mix

Applications

- *in vitro* transcription reactions
- Production of RNA probes and transcripts

Description

Bioline NTPs (Ribonucleoside-5'-tri-phosphates) are manufactured in-house and are tested in functional assays with the Bioline RNA polymerases. Bioline NTPs are >98% pure as analysed by HPLC and are free of DNase, RNase, Protease, Phosphatase and nicking activity.

The NTP Set consists of 4 separate 100mM solutions (ATP, GTP, CTP, and UTP, (pH 7.5)) as sodium salts. Each solution contains 25µmol (250µl) of the corresponding NTP. For *in vitro* RNA synthesis, mix equal volumes of all separate NTP solutions.

The NTP Mix is a solution containing 25µmol of each ATP, GTP, CTP and UTP (pH 7.5) as sodium salts in a convenient mix at 100mM (total NTP concentration).

Storage Conditions

NTPs can be stored for 12 months at -20°C. Avoid multiple freeze/thaw cycles. For long-term usage, aliquoting is recommended.

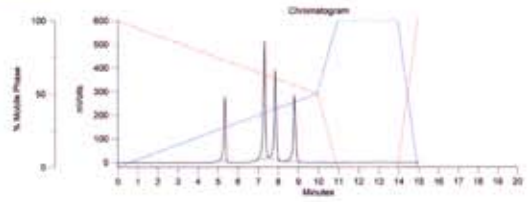
Associated Products

PRODUCT	CAT NO.	CHAPTER
dATP	BIO-39036	Nucleotides
dCTP	BIO-39038	Nucleotides
dGTP	BIO-39037	Nucleotides
dTTP	BIO-39039	Nucleotides

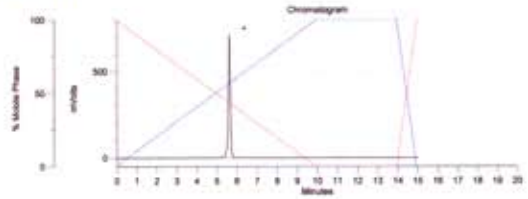
Quality Control

The dNTPs are completely free of modified bases as well as tetraphosphate and pyrophosphate contamination. This high degree of purity is important since contamination can readily inhibit PCR reactions. The attached figures illustrate various assays, which are used to validate the performance and sensitivity of the Bioline dNTPs.)

Mix



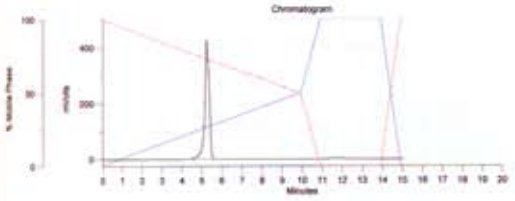
dUTP



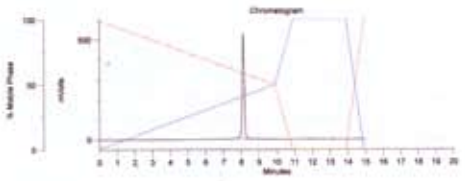
PHYSICAL CONSTANTS, SPECTRAL & HPLC

	dUTP	dCTP	dGTP
Product	dUTP Lithium 100mM Solution	dCTP Lithium 100mM Solution	dGTP Lithium 100mM Solution
Nomenclature	2'-deoxyuridine-5'-triphosphate	2'-deoxycytidine-5'-triphosphate	2'-deoxyguanosine-5'-triphosphate
Formula	$C_9H_{12}N_2O_{14}P_3Li_4$	$C_9H_{12}N_3O_{13}P_3Li_4$	$C_{10}H_{12}N_5O_{13}P_3Li_4$
Molecular Weight	492.864g/mol	490.891g/mol	530.916g/mol
λmax pH 7.0	262nm	272nm	252nm
ε at λmax	10.0 E x $mmol^{-1}$ x cm^{-1}	9.1 E x $mmol^{-1}$ x cm^{-1}	13.7 E x $mmol^{-1}$ x cm^{-1}
A_{250}/A_{260}	0.75 ± 0.03	0.82 ± 0.03	1.16 ± 0.05
A_{280}/A_{260}	0.38 ± 0.02	0.98 ± 0.03	0.66 ± 0.03
Concentration	100mM ± 2%	100mM ± 2%	100mM ± 2%
Appearance	Clear Colourless Solution	Clear Colourless Solution	Clear Colourless Solution
pH of Solution	7.5	7.5	7.5
dNTP (HPLC Area)	≥99%	≥99%	≥99%
dNDP (HPLC Area)	<1%	<1%	<1%
DNases, RNases, Nicking Activity	Negative	Negative	Negative
Storage	at -20°C	at -20°C	at -20°C
Stability	≥12 months	≥12 months	≥12 months

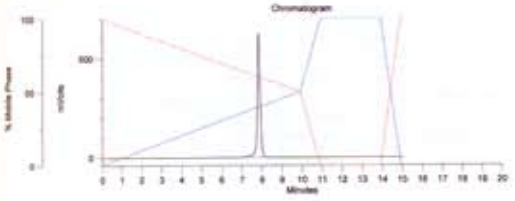
dCTP



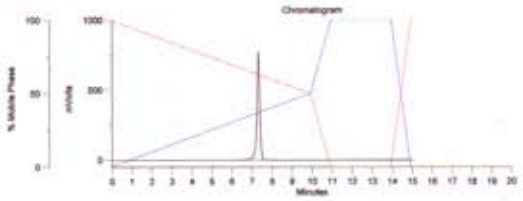
dITP



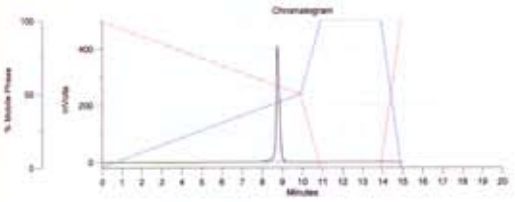
dGTP



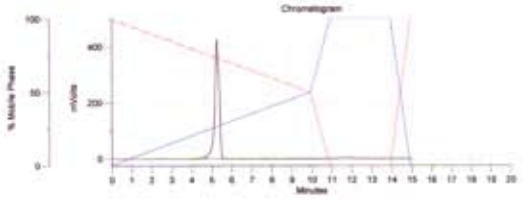
dATP



dTTP



HMdCTP



dTTP	dITP	dATP	HMdCTP
dTTP Lithium 100mM Solution	dITP Lithium 100mM Solution	dATP Lithium 100mM Solution	HMdCTP Lithium 100mM Solution
2'-deoxythymidine-5'-triphosphate	2'-deoxyinosine-5'-triphosphate	2'-deoxyadenosine-5'-triphosphate	5 hydroxymethyl 2'-deoxycytidine-5'-triphosphate
$C_{10}H_{12}N_2O_{14}P_3Li_4$	$C_{10}H_{12}N_4O_{13}P_3Li_4$	$C_{10}H_{12}N_5O_{12}P_3Li_4$	$C11H14N_3O_{14}P_3Li_4$
505.903g/mol	516.909g/mol	514.916g/mol	520.918g/mol
267nm	249nm	259nm	275nm
9.6 E x $mmol^{-1}$ x cm^{-1}	12.2 E x $mmol^{-1}$ x cm^{-1}	15.4 E x $mmol^{-1}$ x cm^{-1}	7.7 E x $mmol^{-1}$ x cm^{-1}
0.65 ± 0.03	1.68 ± 0.04	0.78 ± 0.03	0.90 ± 0.03
0.73 ± 0.02	0.25 ± 0.02	0.15 ± 0.02	1.33 ± 0.03
100mM ± 2%	100mM ± 2%	100mM ± 2%	100mM ± 2%
Clear Colourless Solution	Clear Colourless Solution	Clear Colourless Solution	Clear Colourless Solution
7.5	7.5	7.5	7.5
≥99%	≥99%	≥99%	≥99%
<1%	<1%	<1%	<1%
Negative	Negative	Negative	Negative
at -20°C	at -20°C	at -20°C	at -20°C
≥12 months	≥12 months	≥12 months	≥12 months

DNA Polymerases

Enzymes

Quick Reference Legend:

HF High Fidelity DNA Polymerases | HA Heat-Activated Polymerases | SA Polymerases For Special Applications | *Taq* *Taq* DNA Polymerases

DNA Polymerases

Each polymerase has different characteristics, so it is crucial to choose the enzyme that suits your individual experiment. Bioline polymerases are available alone but are also supplied as convenient ready-to-use 2x Reaction Mixes containing the polymerase of choice plus dNTPs, $MgCl_2$ and further additives to achieve optimal reaction conditions.

The following guides are designed facilitate your selection of polymerases.

Note: Bioline polymerase mixes contain our ultra-pure dNTPs manufactured in-house. Bioline >99% pure nucleotides help you to succeed in your experiments.

Taq DNA POLYMERASES

PRODUCT	APPLICATIONS	ADVANTAGES
BIOTAQ™	Wide Range of Assays	Very robust yield Excellent price/performance
BioMix™	Same as BIOTAQ	Ready-to-use mix Reduced risk of contamination Can be stored at 4°C for 1 month
Mango *Taq*™	Any *Taq* based reactions Suited to high throughput	Direct loading onto Agarose Gels Coloured reaction buffers for easy recognition

HEAT-ACTIVATED POLYMERASES

PRODUCT	Relative Accuracy to *Taq*	APPLICATIONS	ADVANTAGES
IMMOLASE™	Same as *Taq*	Assays requiring a heat-activated assay Highly suited for use in quantitative assays	Robust performance
ImmoMix™ **ImmoMix™Red**	Same as *Taq*	Assays requiring ready-to-use mix	Fewer handling steps Stable at room temperature for prolonged periods
AccuSure™ **AccuSure™ Mix**	Up to 47-Fold Greater	Assays requiring high fidelity and high specificity	Heat-activated with higher fidelity than *Taq* Ready-to-use mix

Polymerase Properties

POLYMERASE	SUITABLE TEMPLATE LENGTH	HEAT ACTIVATED	SENSITIVITY / LOW COPY TEMPLATES	ELONGATION REQUIRED (BASES/SEC)
ACCUZYME™	Up to 5Kb	NO	*	20 - 40
ACCUZYME™ Mix	Up to 5Kb	NO	*	20 - 40
AccuSure™	Up to 5Kb	YES	*	20 - 40
AccuSure™ Mix	Up to 5Kb	YES	*	20 - 40
IMMOLASE™	Up to 5Kb	YES	**	30 - 70
ImmoMix™ / ImmoMix™Red	Up to 5Kb	YES	**	30 - 70
BIO-X-ACT™ Short	Up to 3Kb	NO	**	40 - 60
BIO-X-ACT™ Short Mix	Up to 3Kb	NO	**	40 - 60
BIO-X-ACT™ Long	3 - 20Kb	NO	**	40 - 60
BIO-X-ACT™ Long Mix	3 - 20Kb	NO	**	40 - 60
Diamond	Up to 5Kb	NO	*	20 - 30
BIOTAQ™	Up to 5Kb	NO	**	30 - 70
BioMix™	Up to 5Kb	NO	**	30 - 70
Mango *Taq*™	Up to 5Kb	NO	**	30 - 70

HIGH FIDELITY DNA POLYMERASES

PRODUCT	Relative Accuracy to *Taq*	APPLICATIONS	ADVANTAGES
ACCUZYME™	Up to 47-Fold Greater	Assays requiring very high fidelity Gene cloning	Extremely high yield coupled with high fidelity Optimised buffer for high fidelity Available heat-activated
ACCUZYME™ Mix	Up to 47-Fold Greater	Ready-to-use high fidelity mix	Just add template & primers
AccuSure™	Up to 47-Fold Greater	Extremely high fidelity coupled with high specificity DHPLC compatible	Heat-activated for high specificity Robust performance
AccuSure™ Mix	Up to 47-Fold Greater	Applications requiring minimal handling	Ready-to-use master mix

POLYMERASES FOR SPECIAL APPLICATIONS

PRODUCT	Relative Accuracy to *Taq*	APPLICATIONS	ADVANTAGES
BIO-X-ACT™ Short	Up to 17-Fold Greater	Low copy template Sensitive templates of <3Kb	Suited to difficult templates <3Kb Can process GC-rich templates Higher performance coupled with higher fidelity than *Taq*
BIO-X-ACT™ Short Mix	Up to 17-Fold Greater	Difficult templates requiring minimal handling	Ready-to-use mix
BIO-X-ACT™ Long	Up to 17-Fold Greater	For long and accurate assays of <20Kb genomic templates	Can process genomic template <20Kb Minimal optimisation required
BIO-X-ACT™ Long Mix	Up to 17-Fold Greater	Assays requiring minimal handling	Ready-to-use mix
Diamond	Same as *Taq*	Suited to multiplex reactions Extremely high specificity	Extremely high specificity Suited to SNP detection, genotyping
Diamond Mix	Same as *Taq*	Ideal for high-specificity assays requiring minimal handling	Ready-to-use mix

SPECIFICITY	PROBLEM TEMPLATES	FIDELITY	GC-RICH TEMPLATES	PERFORMANCE	3' END MODIFICATION	YIELD
*	*	***	*	**	Blunt	**
*	*	***	*	**	Blunt	**
**	*	***	*	**	Blunt	**
**	*	***	*	**	Blunt	**
***	*		*	**	3'-dA	***
***	*		*	**	3'-dA	***
**	***	**	*	**	Mix (3'-dA + Blunt)	***
**	***	**	*	**	Mix (3'-dA + Blunt)	***
**	***	**	**	**	Mix (3'-dA + Blunt)	**
**	***	**	**	**	Mix (3'-dA + Blunt)	**
***	***	*	***	*	3'-dA	*
*			*	***	3'-dA	***
*			*	***	3'-dA	***
*			*	**	3'-dA	**

ACCUZYME™ DNA Polymerase

HF

CONC.	PACK SIZE	CAT NO.
2.5u/µl	250 Units	BIO-21051
2.5u/µl	500 Units	BIO-21052

ACCUZYME Includes: 10x AccuBuffer and 50mM $MgCl_2$ solution

Features

- **Extremely high fidelity**
- **High yield**
- **Processes fragments <5Kb**
- **Produces blunt ends**
- **DHPLC compatible (detergent-free)**
- **Available as a convenient pre-mixed, pre-optimised solution (ACCUZYME Mix)**

Applications

- **Ultra-high fidelity cloning**
- **High-throughput**
- **Ultra-high fidelity primer extension**

Highest Fidelity

ACCUZYME™ DNA polymerase and the heat-activated version, AccuSure™ DNA polymerase, offer the highest fidelity of all the Bioline polymerases, with 47-fold greater fidelity than *Taq*.

Exceptional Yield

In contrast to other commercially available polymerases, ACCUZYME exhibits extremely high yield coupled with high fidelity.

DHPLC

ACCUZYME is optimised for use in a detergent-free system, making it ideally suited for direct loading of samples on to DHPLC columns.

Description

ACCUZYME is a thermostable enzyme possessing 5'-3' DNA polymerase and 3'-5' proof-reading exonuclease activities, offering extremely high fidelity (up to 47-fold higher fidelity than *Taq*). ACCUZYME produces blunt-ended amplicons of up to 5Kb in length.

ACCUZYME is supplied with 10x buffer containing $MgSO_4$, which provides optimal final reaction conditions (2mM Mg^{2+}) for most experiments. In order to allow optimisation of reaction conditions, $MgCl_2$ is additionally provided.

Specificity and performance of ACCUZYME can be further improved with the use of 2x PolyMate Additive (not supplied, see associated products), which is designed for GC or AT-rich DNA, "dirty" templates or sequences with difficult melting profiles.

Unit Definition

One unit will incorporate 10nmoles of dNTPs in 30min at 72°C.

Concentration

2.5u/µl

Storage Conditions

ACCUZYME can be stored for 12 months at -20°C.

Note: Purchase of this product does not convey a licence to perform any patented process.

ACCUZYME™ Mix

HF

CONC.	PACK SIZE	CAT NO.
2x	100 Reactions	BIO-25027
2x	100 Reactions	BIO-25028

ACCUZYME Mix includes: 50mM $MgCl_2$ solution

Description

ACCUZYME™ is also available as a convenient 2x Reaction Mix to maximise experiment reproducibility. ACCUZYME™ Mix contains ACCUZYME DNA Polymerase, ultra-pure dNTPs manufactured by Bioline, and $MgCl_2$. The Mix is optimised and ready-to-use: the user adds only water, template and primers.

ACCUZYME Mix is supplied with an additional 50mM of $MgCl_2$ solution for optional optimisation of reaction conditions.

Extended Stability

A sample of ACCUZYME Mix was stored at +20°C over a five-week period and tested daily. No detectable loss of activity was evidenced. However, in view of the possibility of microbial contamination, please adhere to the recommended storage conditions.

Concentration

2x

Storage Conditions

ACCUZYME Mix can be stored for up to 6 months at -20°C, or up to 2 weeks at +4°C.

Note: Purchase of this product does not convey a licence to perform any patented process.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Set	BIO-39025	Nucleotides
dNTP Mix	BIO-39028	Nucleotides
2x PolyMate Additive	BIO-37041	Reagents for Molecular Biology
AccuSure	BIO-21069	Enzymes for Molecular Biology
HyperLadder I	BIO-33025	Molecular Weight Markers
Agarose	BIO-41025	Reagents for Molecular Biology
SureClean Plus	BIO-37048	Reagents for Molecular Biology

AccuSure™ DNA Polymerase

HA HF

CONC.	PACK SIZE	CAT NO.
2.5u/µl	250 Units	BIO-21066
2.5u/µl	500 Units	BIO-21069

AccuSure Includes: 10x AccuBuffer and 50mM $MgCl_2$ solution

Features

- Heat-activated
- High fidelity
- Very high specificity
- Processes fragments <5Kb
- Produces blunt ends
- DHPLC compatible (detergent-free)
- Available as a convenient pre-mixed, pre-optimised solution (AccuSure Mix)

Applications

- High-fidelity applications leading to cloning
- Assays requiring blunt ending
- High-throughput

Highest Fidelity coupled with Heat-activation

AccuSure™ DNA polymerase delivers 47-fold higher fidelity than *Taq* and extremely high specificity owing to its heat-activation. AccuSure is highly suited to challenging assays in which background must be kept to a minimum.

Description

AccuSure possesses the same high fidelity as ACCUZYME, but has the additional advantage of being heat-activated, offering enhanced specificity. AccuSure is ideally suited for difficult templates, such as DNA secondary structures and microsatellites. AccuSure produces blunt-ended amplicons of up to 5Kb in length.

AccuSure is optimised for use in a detergent-free system, making it ideally suited for direct loading of samples on to DHPLC columns.

AccuSure is supplied with 10x buffer containing $MgSO_4$, which provides optimal final reaction conditions (2mM Mg^{2+}) for most experiments. In order to allow optimisation of reaction conditions, $MgCl_2$ is additionally provided.

Specificity and performance of AccuSure can be further improved with the use of 2x PolyMate Additive (not supplied, see associated products), which is designed for GC or AT-rich DNA, "dirty" templates or sequences with difficult melting profiles.

Unit Definition

One unit will incorporate 10nmoles of dNTPs in 30min at 72°C.

Concentration

2.5u/µl

Storage Conditions

AccuSure can be stored for 12 months at -20°C.

Note: Purchase of this product does not convey a licence to perform any patented process.

AccuSure™ Mix

HA HF

CONC.	PACK SIZE	CAT NO.
2x	100 Reactions	BIO-25029
2x	500 Reactions	BIO-25030

AccuSure Mix Includes: 50mM $MgCl_2$ solution

Description

AccuSure is also available as a convenient 2x Reaction Mix to maximise experiment reproducibility. AccuSure™ Mix contains AccuSure DNA Polymerase, ultra-pure dNTPs manufactured by Bioline and $MgCl_2$. The Mix is optimised and ready-to-use: the user adds only water, template and primers.

AccuSure Mix is supplied with an additional 50mM of $MgCl_2$ solution for optional optimisation of reaction conditions.

Extended Stability

A sample of AccuSure Mix was stored at +20°C over a five-week period and tested daily. No detectable loss of activity was evidenced. However, in view of the possibility of microbial contamination, please adhere to the recommended storage conditions.

Concentration

2x

Storage Conditions

AccuSure Mix can be stored for up to 6 months at -20°C, or up to 2 weeks at +4°C.

Note: Purchase of this product does not convey a licence to perform any patented process.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Set	BIO-39025	Nucleotides
dNTP Mix	BIO-39028	Nucleotides
2x PolyMate Additive	BIO-37041	Reagents for Molecular Biology
ACCUZYME	BIO-21052	Enzymes for Molecular Biology
HyperLadder I	BIO-33025	Molecular Weight Markers
Agarose	BIO-41025	Reagents for Molecular Biology
SureClean Plus	BIO-37048	Reagents for Molecular Biology

IMMOLASE™ DNA Polymerase

HA

CONC.	PACK SIZE	CAT NO.
5u/µl	250 Units	BIO-21046
5u/µl	500 Units	BIO-21047
5u/µl	5000 Units	BIO-21048

IMMOLASE Includes: 10x ImmoBuffer and 50mM $MgCl_2$ solution

Features

- Heat-activated
- Highly suited to real-time assays
- Ultra-high specificity
- Leaves 'A' overhang
- Excellent yield in quantitative assays

Applications

IMMOLASE™ DNA polymerase has been developed for assays that require heat activation for enhanced specificity and reduced background. IMMOLASE delivers high and reliable yields, even in assays prone to primer-dimer formation. With problematic quantitative assays, IMMOLASE offers outstanding and robust performance.

Description

IMMOLASE is a heat-activated thermostable DNA polymerase isolated from a novel organism. IMMOLASE provides improved specificity when compared to standard polymerases and can eliminate the presence of non-specifics, such as primer-dimers and mis-primed products. IMMOLASE is inactive at room temperature and therefore, before primer extension, requires activation by heat treatment for 7 minutes. Subsequently, the reaction can be handled according to the user's existing protocols for thermostable DNA polymerases.

Specificity and performance of IMMOLASE can be further improved with the use of 2x PolyMate Additive (not supplied, see associated products), which is designed for GC or AT-rich DNA, "dirty" templates or sequences with difficult melting profiles.

Unit Definition

One unit will incorporate 10nmoles of dNTPs in 30min at 72°C.

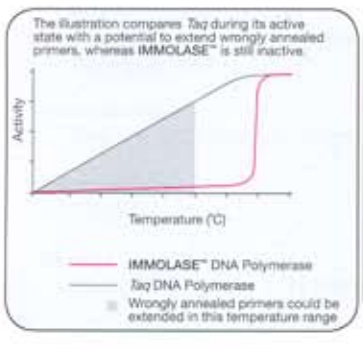
Concentration

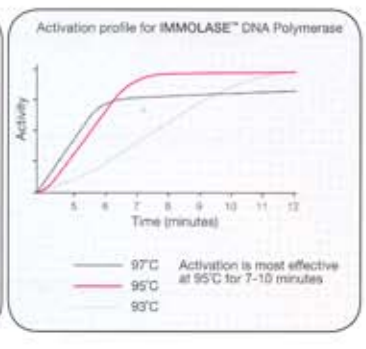
5u/µl

Storage Conditions

IMMOLASE can be stored for 12 months at -20°C.

Note: Purchase of this product does not convey a licence to perform any patented process.





ImmoMix™ & ImmoMix™ Red DNA Polymerase

HA

PRODUCT	CONC.	PACK SIZE	PRICE
ImmoMix	2x	100 Reactions	BIO-25019
ImmoMix	2x	500 Reactions	BIO-25020
ImmoMix Red	2x	100 Reactions	BIO-25021
ImmoMix Red	2x	500 Reactions	BIO-25022

ImmoMix & ImmoMix Red Includes: 50mM $MgCl_2$ solution

Features

- Ready-to-use Reaction Mix
- Heat-activated
- Highly suited to real-time assays
- Reduced risk of contamination

Applications

ImmoMix™ is a pre-mixed reagent and is ideal for laboratories that require reagent conditions to be identical every time. ImmoMix is ideally suited to assays which necessitate a minimum of preparative work.

Description

ImmoMix is a complete ready-to-use heat-activated 2x Reaction Mix, which, to enable the carrying-out of polymerase assays, simply requires the user to add only water, template and primers, and then to pre-heat for 7 minutes. The 7-minute activation step eliminates the presence of non-specifics such as primer-dimers and mis-primed products, by ensuring that the enzyme is inactive at the initial low temperatures.

ImmoMix Red combines all of the features and advantages of ImmoMix, and contains an additional inert red dye, allowing users to load samples directly onto a gel, without the need to add loading buffer since the mix is of sufficiently high density to sink to the bottom of the gel. Adequate mixing is also ensured when reactions are set up.

ImmoMix and ImmoMix Red are based on our IMMOLASE DNA polymerase, which leaves an 'A' overhang, and have been optimised for a wide variety of templates. An additional 50mM of $MgCl_2$ solution is included should any fine adjustments be required.

Extended Stability

A sample of ImmoMix and ImmoMix Red were stored at +20°C over a five-week period and tested daily. No detectable loss of activity was evidenced. However, in view of the possibility of microbial contamination, please adhere to the recommended storage conditions.

Concentration

2x

Storage Conditions

ImmoMix and ImmoMix Red can be stored for up to 6 months at -20°C, or up to 2 weeks at +4°C.

Note: Purchase of this product does not convey a licence to perform any patented process.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Set	BIO-39025	Nucleotides
dNTP Mix	BIO-39028	Nucleotides
2x PolyMate Additive	BIO-37041	Reagents for Molecular Biology
HyperLadder I	BIO-33025	Molecular Weight Markers
Agarose	BIO-41025	Reagents for Molecular Biology
SureClean Plus	BIO-37048	Reagents for Molecular Biology

BIO-X-ACT™ Short DNA Polymerase

SA

CONC.	PACK SIZE	CAT NO.
4u/µl	250 Units	BIO-21064
4u/µl	500 Units	BIO-21065

BIO-X-ACT Short Includes: 10x OptiBuffer, 50mM $MgCl_2$ solution and 5x Hi-Spec Additive

Features

- Ideal for problematic templates
- Ideal for templates that fail with *Taq*
- 17-Fold higher fidelity than *Taq*
- Processes fragments <5Kb
- Leaves 'A' overhang

Description

BIO-X-ACT™ Short DNA polymerase is a high-performance proprietary complex of enzymes specifically designed for difficult/problematic applications requiring high processivity and fidelity. Two versions of BIO-X-ACT are available, for short and long fragments respectively, and are the polymerases of choice for difficult applications that would otherwise fail.

BIO-X-ACT Short is recommended for short Genomic DNA fragments of up to 2Kb, or Lambda DNA fragments up to 5Kb. With Lambda DNA as template, the best performance is achieved within the 100bp to 3Kb range. For longer fragments see BIO-X-ACT™ Long DNA polymerase. BIO-X-ACT Short is ideal for direct cloning without the need to verify the sequence prior to expression. BIO-X-ACT Short possesses 5'-3' polymerase activity and 3'-5' proofreading activity, which in combination with other properties, provides 17-fold higher fidelity than *Taq*.

For enhanced specificity, BIO-X-ACT Short is supplied with a vial of 5x Hi-Spec Additive. Hi-Spec Additive is a very efficient enhancer, which helps to prevent the formation of false background bands and smearing. Specificity and performance can be further improved with the use of 2x PolyMate Additive (not supplied, see associated products), which is designed for GC or AT-rich DNA, "dirty" templates or sequences with difficult melting profiles.

Unit Definition

One unit will incorporate 10nmoles of dNTPs in 30min at 72°C.

Concentration

4u/µl

Storage Conditions

BIO-X-ACT Short can be stored for 12 months at -20°C.

Note: Purchase of this product does not convey a licence to perform any patented process.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Set	BIO-39025	Nucleotides
dNTP Mix	BIO-39028	Nucleotides
2x PolyMate Additive	BIO-37041	Reagents for Molecular Biology
BIO-X-ACT Long	BIO-21050	Enzymes for Molecular Biology
Agarose	BIO-41025	Reagents for Molecular Biology
SureClean Plus	BIO-37048	Reagents for Molecular Biology

BIO-X-ACT™ Short Mix DNA Polymerase

SA

CONC.	PACK SIZE	CAT NO.
2x	100 Reactions	BIO-25025
2x	500 Reactions	BIO-25026

BIO-X-ACT Short Mix Includes: 50mM $MgCl_2$ solution

Features

- Ready-to-use Reaction Mix
- Reduced risk of contamination
- Developed for ease of use
- Processes fragments <5Kb

Description

BIO-X-ACT™ Short Mix is a complete ready-to-use 2x Reaction Mix, which, to enable the carrying-out of polymerase assays on problematic templates, simply requires the user to add water, template and primers. BIO-X-ACT Short Mix has all of the features of our BIO-X-ACT Short DNA polymerase and is suitable for genomic targets of up to 3Kb or lambda targets of up to 5Kb. The mix has been optimised for a wide variety of templates, and an additional 50mM of $MgCl_2$ solution is included should any fine adjustments be required.

BIO-X-ACT Short Mix dramatically reduces the time needed to set up reactions, thereby minimising the risk of contamination. Greater reproducibility is ensured, by reducing the number of pipetting steps that can lead to pipetting errors. All mixes have been tested for scalability, and the mix, (when used at 2x concentration), can be used in reaction volumes from 5µl upwards.

Extended Stability

A sample of BIO-X-ACT Short Mix was stored at +20°C over a five-week period and tested daily. No detectable loss of activity was evidenced. However, in view of the possibility of microbial contamination, please adhere to the recommended storage conditions.

Concentration

2x

Storage Conditions

BIO-X-ACT Short Mix can be stored for up to 6 months at -20°C, or up to 2 weeks at +4°C.

Note: Purchase of this product does not convey a licence to perform any patented process.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Set	BIO-39025	Nucleotides
dNTP Mix	BIO-39028	Nucleotides
2x PolyMate Additive	BIO-37041	Reagents for Molecular Biology
BIO-X-ACT Long	BIO-21050	Enzymes for Molecular Biology
Agarose	BIO-41025	Reagents for Molecular Biology
SureClean Plus	BIO-37048	Reagents for Molecular Biology

BIO-X-ACT™ Long DNA Polymerase

SA

CONC.	PACK SIZE	CAT NO.
4u/µl	250 Units	BIO-21049
4u/µl	500 Units	BIO-21050

BIO-X-ACT Long includes: 10x OptiBuffer, 50mM $MgCl_2$ solution and 5x Hi-Spec Additive

Features

- Ideal for problematic templates
- Ideal for templates that fail with *Taq*
- 17-Fold higher fidelity than *Taq*
- Processes fragments <30Kb
- Leaves 'A' overhang

Description

BIO-X-ACT™ Long DNA polymerase is a high-performance proprietary complex of enzymes specifically designed for difficult/problematic applications requiring high processivity and fidelity. Two versions of BIO-X-ACT are available, for short and long fragments respectively, and are the polymerases of choice for difficult applications that would otherwise fail.

BIO-X-ACT Long is recommended for long Genomic DNA fragments of up to 20Kb, or Lambda DNA fragments up to 30Kb. With Lambda DNA as template, the best performance is achieved within the 2Kb-20Kb range. BIO-X-ACT Long is ideal for direct cloning without the need to verify the sequence prior to expression. BIO-X-ACT Long possesses 5'-3' polymerase activity and 3'-5' proofreading activity, which in combination with other properties, provides 17-fold higher fidelity than *Taq*.

For enhanced specificity, BIO-X-ACT Long is supplied with a vial of 5x Hi-Spec Additive. Hi-Spec Additive is a very efficient enhancer, which helps to prevent the formation of false background bands and smearing. Specificity and performance can be further improved with the use of 2x PolyMate Additive (not supplied, see associated products), which is designed for GC or AT-rich DNA, "dirty" templates or sequences with difficult melting profiles.

Unit Definition

One unit will incorporate 10nmoles of dNTPs in 30min at 72°C.

Concentration

4u/µl

Storage Conditions

BIO-X-ACT Long can be stored for 12 months at -20°C.

Note: Purchase of this product does not convey a licence to perform any patented process.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Set	BIO-39025	Nucleotides
dNTP Mix	BIO-39028	Nucleotides
2x PolyMate Additive	BIO-37041	Reagents for Molecular Biology
BIO-X-ACT Short	BIO-21065	Enzymes for Molecular Biology
Agarose	BIO-41025	Reagents for Molecular Biology
SureClean Plus	BIO-37048	Reagents for Molecular Biology

BIO-X-ACT™ Long Mix DNA Polymerase

SA

CONC.	PACK SIZE	CAT NO.
2x	100 Units	BIO-25023
2x	500 Units	BIO-25024

BIO-X-ACT Long Mix includes: 50mM $MgCl_2$ solution

Features

- Ready-to-use Reaction Mix
- Reduced risk of contamination
- Developed for ease of use
- Processes fragments <15Kb

Description

BIO-X-ACT™ Long Mix DNA polymerase is a complete ready-to-use 2x Reaction Mix, which, to enable the carrying-out of polymerase assays on problematic templates, simply requires the user to add water, template and primers. BIO-X-ACT Long Mix has all of the features of our BIO-X-ACT Long DNA polymerase, and has been tested on Lambda DNA fragments of up to 15Kb. The mix has been optimised for a wide variety of templates, and an additional 50mM of $MgCl_2$ solution is included should any fine adjustments be required.

BIO-X-ACT Long Mix dramatically reduces the time needed to set up reactions, thereby minimising the risk of contamination. Greater reproducibility is ensured, by reducing the number of pipetting steps that can lead to pipetting errors.

All mixes have been tested for scalability, and the mix, (when used at 2x concentration), can be used in reaction volumes from 5µl upwards. For fragments in excess of 15Kb, we recommend using BIO-X-ACT Long (Cat No.BIO-21050) rather than BIO-X-ACT Long Mix. This permits greater flexibility of reaction conditions, which may be required for optimisation of long template reactions.

Extended Stability

A sample of BIO-X-ACT Long Mix was stored at +20°C over a five-week period and tested daily. No detectable loss of activity was evidenced. However, in view of the possibility of microbial contamination, please adhere to the recommended storage conditions.

Concentration

2x

Storage Conditions

BIO-X-ACT Long Mix can be stored for up to 6 months at -20°C, or up to 2 weeks at +4°C.

Note: Purchase of this product does not convey a licence to perform any patented process.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Set	BIO-39025	Nucleotides
dNTP Mix	BIO-39028	Nucleotides
2x PolyMate Additive	BIO-37041	Reagents for Molecular Biology
BIO-X-ACT Short	BIO-21065	Enzymes for Molecular Biology
Agarose	BIO-41025	Reagents for Molecular Biology
SureClean Plus	BIO-37048	Reagents for Molecular Biology

Diamond DNA Polymerase

SA

CONC.	PACK SIZE	CAT NO.
5u/µl	250 Units	BIO-21058
5u/µl	500 Units	BIO-21059

Diamond includes: 10x NH_4 Buffer and 50mM $MgCl_2$ solution

Features

- Extremely high specificity
- Mis-matched regions not extended
- Ideal for GC-rich templates
- Elimination of artefacts
- Suitable for MALDI-TOF spectrometry
- Available as a convenient pre-mixed, pre-optimised solution (Diamond Mix)

Applications

- Multiplex reactions
- Genotyping
- SNP detection
- High-throughput

Description

Diamond DNA Polymerase is designed for difficult templates such as high GC% regions and microsatellites. When templates are challenging, the enzyme maintains excellent specificity and minimal background, even in conditions with high concentrations of Mg^{2+}, primers and dNTPs. On genomic templates, Diamond can be used in the presence of $MgCl_2$ concentrations as high as 10mM. Diamond also extends through inverted tandem repeats and regions with high amounts of secondary structure. Diamond only extends perfectly aligned primers, so that mis-match extension is negligible. Diamond is highly specific and consequently conditions should be determined carefully for each template.

Specificity and performance of Diamond can be further improved with the use of 2x PolyMate Additive (not supplied, see associated products), which is designed for GC or AT-rich DNA, "dirty" templates or sequences with difficult melting profiles.

Unit Definition

One unit will incorporate 10nmoles of dNTPs in 30min at 72°C.

Concentration

5u/µl

Storage Conditions

Diamond can be stored for 12 months at -20°C.

Note: Purchase of this product does not convey a licence to perform any patented process.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Set	BIO-39025	Nucleotides
dNTP Mix	BIO-39028	Nucleotides
2x PolyMate Additive	BIO-37041	Reagents for Molecular Biology
BIO-X-ACT Short	BIO-21065	Enzymes for Molecular Biology
HyperLadder I	BIO-33025	Molecular Weight Markers
Agarose	BIO-41025	Reagents for Molecular Biology
SureClean Plus	BIO-37048	Reagents for Molecular Biology

Diamond Mix

SA

CONC.	PACK SIZE	CAT NO.
2x	100 Reactions	BIO-25031
2x	500 Reactions	BIO-25032

Diamond Mix includes: 10x NH_4 Buffer and 50mM $MgCl_2$ solution

Description

Diamond is also available as a convenient 2x Reaction Mix to maximise experimental reproducibility. Diamond Mix contains Diamond DNA Polymerase, ultra-pure dNTPs manufactured by Bioline, and $MgCl_2$. The Mix is optimised and ready-to-use: the user adds only water, template and primers.

For optional optimisation of reaction conditions, Diamond Mix is supplied with an additional 50mM of $MgCl_2$ solution.

Extended Stability

A sample of Diamond Mix was stored at +20°C over a five-week period and tested daily. No detectable loss of activity was evidenced. However, in view of the possibility of microbial contamination, please adhere to the recommended storage conditions.

Concentration

2x

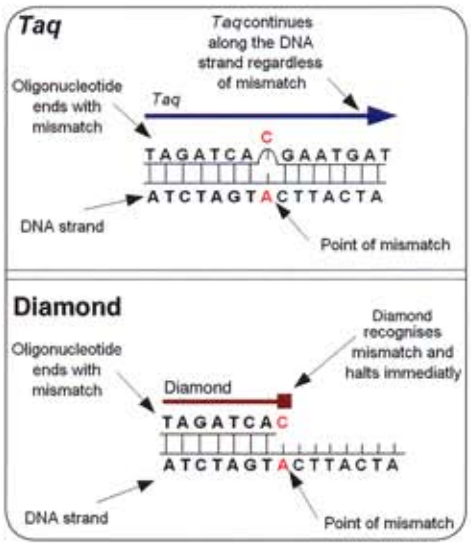
Storage Conditions

Diamond Mix can be stored for up to 6 months at -20°C, or up to 2 weeks at +4°C.

Note: Purchase of this product does not convey a licence to perform any patented process.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Set	BIO-39025	Nucleotides
dNTP Mix	BIO-39028	Nucleotides
2x PolyMate Additive	BIO-37041	Reagents for Molecular Biology
BIO-X-ACT Short	BIO-21065	Enzymes for Molecular Biology
HyperLadder I	BIO-33025	Molecular Weight Markers
Agarose	BIO-41025	Reagents for Molecular Biology
SureClean Plus	BIO-37048	Reagents for Molecular Biology



BIOTAQ™ DNA Polymerase

CONC.	PACK SIZE	CAT NO.
5u/µl	500 Units	BIO-21040
5u/µl	2500 Units	BIO-21060

BIOTAQ includes: 10x NH_4 Buffer and 50mM $MgCl_2$ solution

Features

- Highly purified *Taq* preparation
- Consistent results
- Robust performance
- Wide range of applications
- Leaves an 'A' overhang
- Ideally suited to high-throughput applications

Description

BIOTAQ™ DNA polymerase is a highly purified thermostable DNA polymerase offering very high yield over a wide range of templates, and is the ideal choice for most assays. BIOTAQ is a robust preparation and consistently delivers high yields with minimal background. BIOTAQ possesses 5'-3' exonuclease activity and leaves an 'A' overhang such that the primer extension product is suitable for effective integration into TA cloning vectors.

BIOTAQ is supplied with 10x NH_4-based reaction buffer, which provides optimal conditions for the enzyme, and a separate solution of 50mM $MgCl_2$, allowing reaction conditions to be adjusted to suit the template.

Specificity and performance of BIOTAQ can be further improved with the use of 2x PolyMate Additive (not supplied, see associated products), which is designed for GC or AT-rich DNA, "dirty" templates or sequences with difficult melting profiles.

Unit Definition

One unit will incorporate 10nmoles of dNTPs in 30min at 72°C.

Concentration

5u/µl

Storage Conditions

BIOTAQ can be stored for 12 months at -20°C.

Note: Purchase of this product does not convey a licence to perform any patented process.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Set	BIO-39025	Nucleotides
dNTP Mix	BIO-39028	Nucleotides
2x PolyMate Additive	BIO-37041	Reagents for Molecular Biology
HyperLadder I	BIO-33025	Molecular Weight Markers
SureClean Plus	BIO-37048	Reagents for Molecular Biology

BIOTAQ™ Red DNA Polymerase

CONC.	PACK SIZE	CAT NO.
1u/µl	500 Units	BIO-21041
1u/µl	2500 Units	BIO-21061

BIOTAQ Red includes: 10x NH_4 Buffer and 50mM $MgCl_2$ solution

Features

- Same high performance as BIOTAQ DNA Polymerase
- Direct loading onto agarose gels
- Easy visual recognition
- Ideally suited to high-throughput applications

Description

BIOTAQ™ Red DNA polymerase is a formulation of our regular BIOTAQ, which contains a non-toxic and non-hazardous red dye. The red dye provides easy and quick identification of reactions to which the enzyme has been added, and facilitates the confirmation of complete mixing. When the reaction is complete, a sample of the reaction mix can be loaded directly onto the agarose gel without the need for loading buffer, since the mix is of sufficiently high density to sink to the bottom of the gel. The red dye migrates towards the positive electrode, thereby providing a means to monitor the progress of the electrophoresis.

The presence of the dye has no effect on routine enzymatic manipulations, although rare exceptions may occur.

Specificity and performance of BIOTAQ Red can be further improved with the use of 2x PolyMate Additive (not supplied, see associated products), which is designed for GC or AT-rich DNA, "dirty" templates or sequences with difficult melting profiles.

Unit Definition

One unit will incorporate 10nmoles of dNTPs in 30min at 72°C.

Concentration

1u/µl

Storage Conditions

BIOTAQ Red can be stored for 12 months at -20°C.

Note: Purchase of this product does not convey a licence to perform any patented process.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Set	BIO-39025	Nucleotides
dNTP Mix	BIO-39028	Nucleotides
2x PolyMate Additive	BIO-37041	Reagents for Molecular Biology
HyperLadder I	BIO-33025	Molecular Weight Markers
SureClean Plus	BIO-37048	Reagents for Molecular Biology

BIOTAQ™ Core Kit

Taq

CONC.	PACK SIZE	CAT NO.
5u/µl	500 Units	BIO-21071

Features

- Set of optimised components
- Contains Ultra-pure dNTPs manufactured by Bioline
- Ideal for setting up new procedures

Description

The BIOTAQ™ Core Kit contains all the necessary components to perform polymerase assays on a wide range of DNA templates. In addition to dNTPs, the Core Kit is based on our widely used BIOTAQ DNA polymerase, which achieves dependable and robust results.

Kit components are as follows:

- BIOTAQ DNA Polymerase
- 10x NH_4 Buffer
- 50mM $MgCl_2$ solution
- 40mM dNTP Mix
- 2x PolyMate Additive

Unit Definition

One unit will incorporate 10nmoles of dNTPs in 30min at 72°C.

Concentration

5u/µl

Storage Conditions

BIOTAQ Core Kit can be stored for 12 months at -20°C.

Note: Purchase of this product does not convey a licence to perform any patented process.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Set	BIO-39025	Nucleotides
HyperLadder I	BIO-33025	Molecular Weight Markers
SureClean Plus	BIO-37048	Reagents for Molecular Biology

BioMix™ and BioMix™ Red

Taq

PRODUCT	CONC.	PACK SIZE	CAT NO.
BioMix	2x	100 Reactions	BIO-25011
BioMix	2x	500 Reactions	BIO-25012
BioMix Red	2x	100 Reactions	BIO-25005
BioMix Red	2x	500 Reactions	BIO-25006

BioMix & BioMix Red includes: 50mM $MgCl_2$ solution

Features

- Same high performance as BIOTAQ
- Convenient, ready-to-use
- Reduced risk of contamination
- Reproducible results
- Pre-mixed, pre-optimised solutions

Description

BioMix™ is a complete ready-to-use 2x Reaction Mix containing BIOTAQ DNA polymerase, which, to enable the carrying-out of Polymerase assays of many common genomic and cDNA templates, simply requires the user to add water, template and primers. BioMix dramatically reduces the time required to set up reactions, thereby minimising the risk of contamination. Greater reproducibility is ensured, by reducing the number of pipetting steps that can lead to errors.

BioMix Red combines all of the features and advantages of BioMix, and contains an additional inert red dye that permits easy visualisation and direct loading onto a gel, without the need to add loading buffer, since the mix is of sufficiently high density to sink to the bottom of the gel.

BioMix and BioMix Red have been optimised for a wide variety of templates; however an additional 50mM of $MgCl_2$ solution is included should any fine adjustments be required.

Specificity and performance of BioMix and BioMix Red can be further improved with the use of 2x PolyMate Additive (not supplied, see associated products), which is designed for GC or AT-rich DNA, "dirty" templates or sequences with difficult melting profiles.

Extended Stability

A sample of BioMix and BioMix Red was stored at +20°C over a five-week period and tested daily. No detectable loss of activity was evidenced. However, in view of the possibility of microbial contamination, please adhere to the recommended storage conditions.

Concentration

2x

Storage Conditions

BioMix and BioMix Red can be stored for up to 6 months at -20°C, or up to 2 weeks at +4°C.

Note: Purchase of this product does not convey a licence to perform any patented process.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Mix	BIO-39028	Nucleotides
2x PolyMate Additive	BIO-37041	Reagents for Molecular Biology
HyperLadder I	BIO-33025	Molecular Weight Markers
SureClean Plus	BIO-37048	Reagents for Molecular Biology

MangoTaq™ DNA Polymerase

Taq

CONC.	PACK SIZE	CAT NO.
1u/µl	1000 Units	BIO-21083
1u/µl	2000 Units	BIO-21082
1u/µl	5000 Units	BIO-21078

MangoTaq includes: 10x NH_4 based MangoTaq Buffer and 50mM $MgCl_2$ solution

Features

- Robust performance
- Reaction Buffer contains inert red and orange dyes
- Direct gel-loading
- Leaves 'A' overhang
- Excellent price/performance
- High-throughput applications
- Separate $MgCl_2$ supplied

Description

MangoTaq™ DNA Polymerase is a new Bioline formulation of *Taq* DNA Polymerase, and is supplied with a coloured 10x Reaction Buffer which contains two inert dyes. The Red and Orange dyes separate during gel electrophoresis and provide quick reference points for monitoring the mobility of the DNA samples in the gel.

The reaction mixture containing the inert Red and Orange dyes can be loaded directly onto an agarose gel for analysis, without the need for separate gel-loading buffer.

MangoTaq offers consistent results across a wide range of DNA templates and also leaves an 'A' overhang such that the primer-extension product is suitable for effective integration into TA cloning vectors. MangoTaq is an ideal choice for high-throughput applications, since it combines a lower concentration of *Taq* (1u/µl), with inert coloured dyes to facilitate easy recognition.

Unit Definition

One unit will incorporate 10nmoles of dNTPs in 30min at 72°C.

Concentration

1u/µl

Storage Conditions

MangoTaq can be stored for 12 months at -20°C.

Note: Purchase of this product does not convey a licence to perform any patented process.

TBE Buffer
(5, 10, 15 and 20 µl)

Red and orange dyes after electrophoresis. Differing volumes of the amplification reactions subjected to electrophoresis.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Set	BIO-39025	Nucleotides
dNTP Mix	BIO-39028	Nucleotides
2x PolyMate Additive	BIO-37041	Reagents for Molecular Biology
IMMOLASE	BIO-21048	Enzymes for Molecular Biology
HyperLadder I	BIO-33025	Molecular Weight Markers
Agarose	BIO-41025	Reagents for Molecular Biology
SureClean Plus	BIO-37048	Reagents for Molecular Biology

T4 DNA Polymerase

CONC.	PACK SIZE	CAT NO.
10u/µl	250 Units	BIO-27034
10u/µl	500 Units	BIO-27035

T4 Includes: 10x Reaction Buffer

Features

- 5'-3' DNA polymerase activity
- 3'-5' proof-reading exonuclease activity
- Lacks 5'-3' exonuclease activity

Applications

- *in vitro* mutagenesis
- Probe-labelling
- Removal of 3' overhangs to form blunt-ends

Description

T4 DNA Polymerase catalyses the synthesis of DNA in the 5'-3' direction in the presence of template and primers. This enzyme has 3'-5' proof-reading exonuclease activity.

Unit Definition

One unit will incorporate 10nmoles of dNTPs in 30min at 72°C.

Concentration

10u/µl

Storage Conditions

T4 DNA Polymerase can be stored for 12 months at -20°C.

Note: Purchase of this product does not convey a licence to perform any patented process.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Set	BIO-39025	Nucleotides
dNTP Mix	BIO-39028	Nucleotides
2x PolyMate Additive	BIO-37041	Reagents for Molecular Biology
HyperLadder I	BIO-33025	Molecular Weight Markers
SureClean Plus	BIO-37048	Reagents for Molecular Biology

Klenow Fragment

PRODUCT	PRESENTATION	CAT NO.
5u/µl	500 Units	BIO-27029

Features

- Produces blunt ends
- Lacks exonuclease activity
- Sequencing grade

Applications

- Second strand cDNA synthesis
- Filling-in 5' Ends
- Radio labelling and dideoxy sequencing

Description

Klenow fragment is 68KDa proteolytic subfragement of *E. coli* DNA polymerase I, obtained by subtilising cleavage of the holenzyme. The enzyme is purified from *E. coli* PVG-AI strain.

Unit Definition

One unit will incorporate 10nmoles of dNTPs in 30min at 72°C.

Concentration

5u/µl

Storage Conditions

Klenow Fragment can be stored for 12 months at -20°C.

Note: Purchase of this product does not convey a licence to perform any patented process.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Mix	BIO-39028	Nucleotides
2x PolyMate Additive	BIO-37041	Reagents for Molecular Biology
HyperLadder I	BIO-33025	Molecular Weight Markers
SureClean Plus	BIO-37048	Reagents for Molecular Biology

Uracil DNA Glycosylase (UDG)

CONC.	PACK SIZE	CAT NO.
1u/µl	500 Units	BIO-27044

Features

- Recombinant source
- DNase/RNase-free
- Supplied with 10x Reaction Buffer

Application

- Removal of Uracil from Uracil-containing DNA

Description

Uracil DNA Glycosylase (UDG) catalyses the release of uracil from uracil-containing single-stranded or double-stranded DNA, but not from RNA.

Unit Definition

One unit is the amount of enzyme that catalyses the release of 60µmol of Uracil per minute from Uracil-containing double-stranded DNA.

Concentration

1u/µl

Storage Conditions

Uracil DNA Glycosylase can be stored for 6 months at -20°C.

Note: Purchase of this product does not convey a licence to perform any patented process.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dUTP	BIO-39035	Nulceotides

Introduction to DNA Markers

HyperLadders and EasyLadders

Bioline offers a wide range of DNA Ladders with Loading Buffer in a single tube, enabling accurate sizing and quantitation of DNA ranging between 25bp and 48500bp. The ready-to-use format minimises the time spent thawing, diluting and adding tracking dye to the DNA Ladder. Simply transfer the Ladder from the vial to the gel. An additional 5x Sample Loading Buffer is supplied for your convenience.

DNA Ladders are nucleic acid fragments of specific base pair length, designed for sizing linear double-stranded DNA fragments. Bioline ready-to-use DNA HyperLadders are specifically created for accurate quantitation of DNA fragments in gels, and each ladder includes one, two or three higher-intensity reference bands for easy identification and orientation. HyperLadders are supplied premixed with loading buffer and are stable at room temperature. There is no need to heat, mix or dilute HyperLadders prior to loading them onto a gel.

EasyLadders contain all the features of our HyperLadder range, but are designed for short runs (1 to 3cm) in standard or high-throughput agarose gels, providing a fast way to determine size and quantity of DNA fragments.

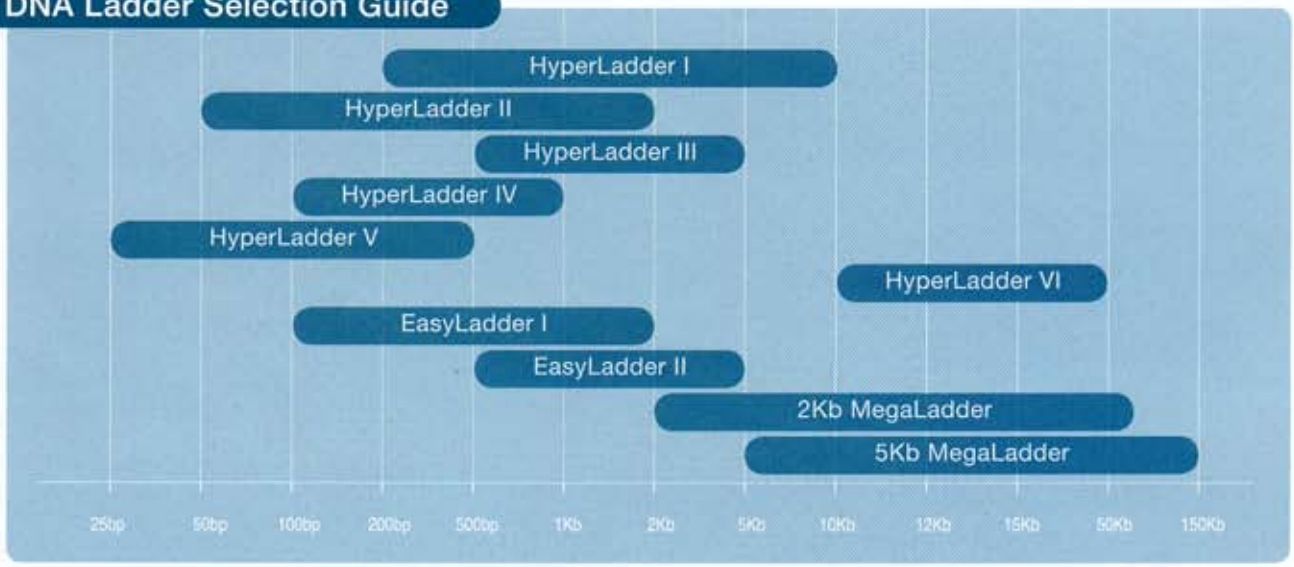
Storage Conditions

HyperLadders and EasyLadders should be stored at -20°C until first use and thereafter at +4°C for up to 6 months. Avoid multiple freeze/thaw cycles as these can damage the product.

Separation of DNA Ladders

LADDER	SEPARATION RANGE (bp)	LEADING DYE COLOR	HIGH INTENSITY BANDS (bp)
HyperLadder I	200 - 10000	Blue	1000 & 10000
HyperLadder II	50 - 2000	Blue	300, 1000 & 2000
HyperLadder III	500 - 5000	Red	1000 & 3500
HyperLadder IV	100 - 1000	Blue	1000
HyperLadder V	25 - 500	Blue	100 & 200
HyperLadder VI	10090 - 48000	Blue	38420
EasyLadder I	100 - 2000	Red	Even Bands
EasyLadder II	500 - 5000	Red	Even Bands
MegaLadder 2Kb	2000 - 80000	Blue	40000
MegaLadder 5Kb	5000 - 150000	Blue	40000

DNA Ladder Selection Guide



MegaLadders

MegaLadders are markers for DNA of high molecular weight, and are designed to enable the accurate sizing of DNA between 2Kb and 80Kb (2Kb MegaLadder) or 5Kb and 150Kb (5Kb MegaLadder). A band at 40Kb has the highest intensity for easy identification and orientation. For the superior resolution of fragments above 30Kb, the use of Pulse Field Inversion Electrophoresis is recommended. No agarose blocks are required for PFI electrophoresis. A separate 6x Sample Loading Buffer is supplied for added convenience.

Storage Conditions

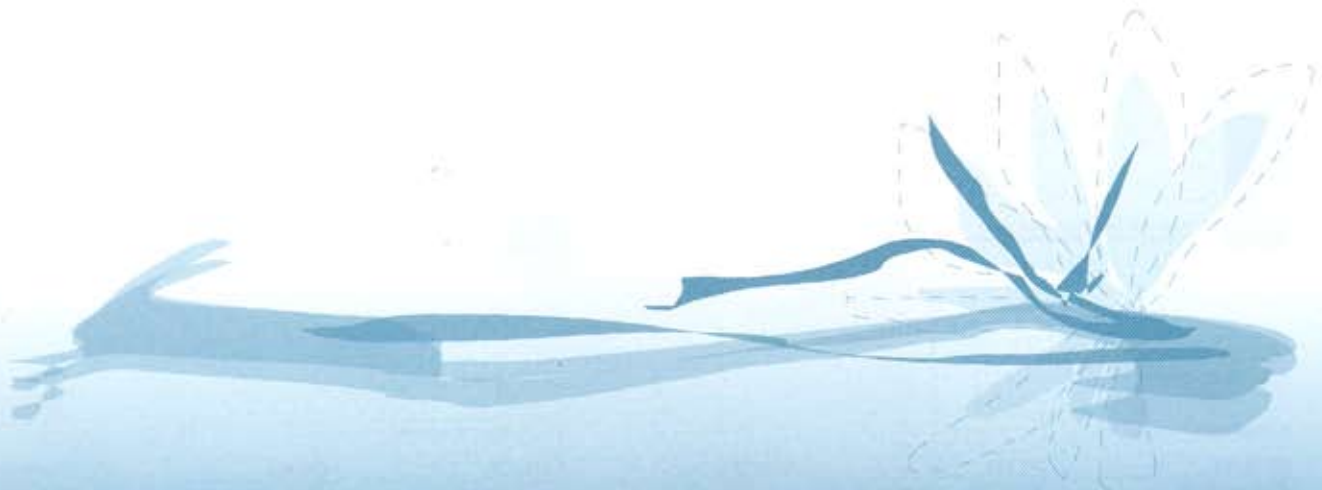
MegaLadders can be stored for 12 months at -20°C.

Coloured DNA Loading Buffers

Bioline has a range of coloured DNA Loading Buffers (Blue, Red and Tri-Colour) based on Bromophenol Blue. The coloured DNA Loading Buffers allow users to monitor DNA migration and therefore by choosing the buffer suitable to their application, increase the versatility of their DNA analysis.

Coloured DNA Loading Buffer Dye Migration

AGAROSE	RED	BLUE
0.7%	1500bp	900bp
1.0%	750bp	400bp
1.5%	500bp	250bp
2.0%	250bp	100bp
3.0%	75bp	30bp



HyperLadder I

PRESENTATION	PACK SIZE	CAT NO.
2 x 500µl	200 Lanes	BIO-33025
5 x 500µl	500 Lanes	BIO-33026

HyperLadder I Includes: 5x Sample Loading Buffer

Size and Quantify DNA fragments from 200bp to 10000bp

Features

- 14 bands (200bp - 10000bp)
- Accurate quantitation
- Easy identification and orientation
- Ready-to-use format

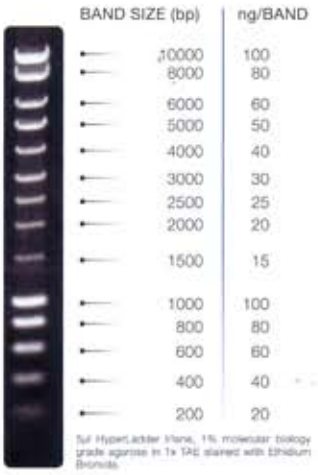
Description

A popular DNA Ladder with loading buffer in a single tube, enabling accurate sizing and quantitation of DNA between 200bp and 10000bp. Each band contains an exact amount of dsDNA: 5µl per lane gives a total of 720ng DNA. Bands at 1000bp and 10000bp have the highest intensity for easy identification and orientation. This ready-to-use format reduces handling steps and saves time; simply transfer HyperLadder I from the vial to the gel.

Storage Conditions

HyperLadder I should be stored at -20°C until first use and thereafter at +4°C for up to 6 months. Avoid multiple freeze/thaw cycles.

HyperLadder I



5µl HyperLadder I/lane, 1% molecular biology grade agarose in 1x TAE stained with Ethidium Bromide.

Associated Products

PRODUCT	CAT NO.	CHAPTER
Agarose Tablets	BIO-41028	Reagents for Molecular Biology
Coloured DNA Loading Buffers	BIO-37070	Reagents for Molecular Biology

HyperLadder II

PRESENTATION	PACK SIZE	CAT NO.
2 x 500µl	200 Lanes	BIO-33039
5 x 500µl	500 Lanes	BIO-33040

HyperLadder II Includes: 5x Sample Loading Buffer

Size and Quantify DNA fragments from 50bp to 2000bp

Features

- 15 bands (50bp - 2000bp)
- Accurate quantitation
- Easy identification and orientation
- Ready-to-use format

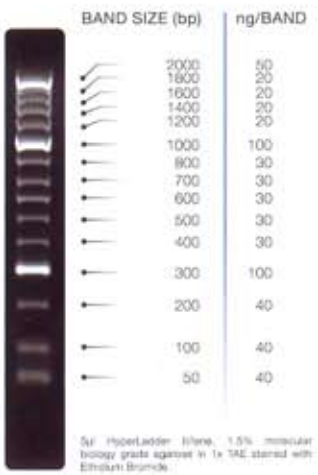
Description

A DNA Ladder with loading buffer in a single tube, enabling accurate sizing and quantitation of DNA between 50bp and 2000bp. Each band contains an exact amount of DNA: 5µl per lane gives a total of 600ng DNA. Bands at 300bp, 1000bp and 2000bp have the highest intensity for easy identification and orientation. This ready-to-use format reduces handling steps and saves time; simply transfer HyperLadder II from the vial to the gel.

Storage Conditions

HyperLadder II should be stored at -20°C until first use, and thereafter at +4°C for up to 6 months. Avoid multiple freeze/thaw cycles.

HyperLadder II



5µl HyperLadder II/lane, 1.5% molecular biology grade agarose in 1x TAE stained with Ethidium Bromide.

Associated Products

PRODUCT	CAT NO.	CHAPTER
Agarose Tablets	BIO-41028	Reagents for Molecular Biology
Coloured DNA Loading Buffers	BIO-37070	Reagents for Molecular Biology

HyperLadder III

PRESENTATION	PACK SIZE	CAT NO.
2 x 500µl	200 Lanes	BIO-33043
5 x 500µl	500 Lanes	BIO-33044

HyperLadder III Includes: 5x Sample Loading Buffer

Size and Quantify DNA fragments from 500bp to 5000bp

Features

- 11 bands (500bp - 5000bp)
- Accurate quantitation
- Contains Red dye, migrating at 500bp in 1.5% agarose gel
- Easy identification and orientation
- Ready-to-use format

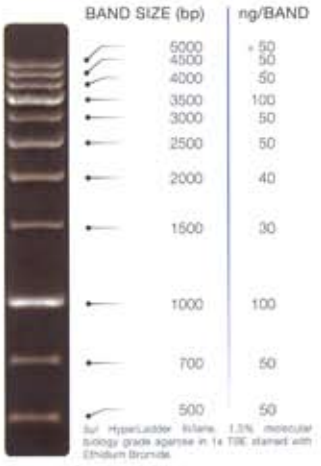
Description

A DNA Ladder with loading buffer in a single tube, enabling accurate sizing and quantitation of DNA between 500bp and 5000bp. Each band contains an exact amount of DNA: 5µl per lane gives a total of 620ng of DNA. Bands at 1000bp and 3500bp have the highest intensity for easy identification and orientation. This ready-to-use format reduces handling steps and saves time; simply transfer HyperLadder III from the vial to the gel.

Storage Conditions

HyperLadder III should be stored at -20°C until first use, and thereafter at +4°C for up to 6 months. Avoid multiple freeze/ thaw cycles.

HyperLadder III



5µl HyperLadder IV/lane, 2% molecular biology grade agarose in 1x TAE stained with Ethidium Bromide.

Associated Products

PRODUCT	CAT NO.	CHAPTER
Agarose Tablets	BIO-41028	Reagents for Molecular Biology
Coloured DNA Loading Buffers	BIO-37070	Reagents for Molecular Biology

HyperLadder IV (IMPROVED FORMULATION)

PRESENTATION	PACK SIZE	CAT NO.
2 x 500µl	200 Lanes	BIO-33029
5 x 500µl	500 Lanes	BIO-33030

HyperLadder IV Includes: 5x Sample Loading Buffer

Size and Quantify DNA fragments from 100bp to 1000bp

Features

- 10 bands (100bp - 1000bp)
- Accurate quantitation
- Easy identification and orientation
- Ready-to-use format

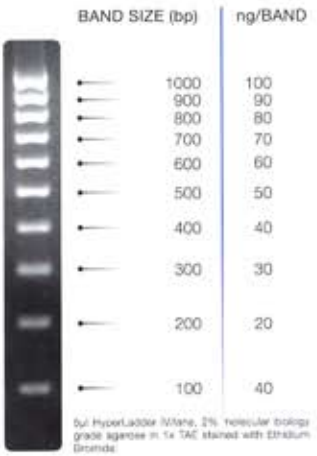
Description

A popular DNA Ladder with loading buffer in a single tube, enabling accurate sizing and quantitation of dsDNA between 100bp and 1000bp. Each band contains an exact amount of DNA: 5µl per lane gives a total of 580ng of dsDNA. This ready-to-use format reduces handling steps and saves time; simply transfer HyperLadder IV from the vial to the gel.

Storage Conditions

HyperLadder IV should be stored at -20°C until first use, and thereafter at +4°C for up to 6 months. Avoid multiple freeze/ thaw cycles.

HyperLadder IV



5µl HyperLadder IV/lane, 2% molecular biology grade agarose in 1x TAE stained with Ethidium Bromide.

Associated Products

PRODUCT	CAT NO.	CHAPTER
Agarose Tablets	BIO-41028	Reagents for Molecular Biology
Coloured DNA Loading Buffers	BIO-37070	Reagents for Molecular Biology

HyperLadder V

PRESENTATION	PACK SIZE	CAT NO.
2 x 500µl	200 Lanes	BIO-33031
5 x 500µl	500 Lanes	BIO-33032

HyperLadder V includes: 5x Sample Loading Buffer

Size and Quantify DNA fragments from 25bp to 500bp

Features

- 12 bands (25bp - 500bp)
- Accurate quantitation
- Easy identification and orientation
- Ready-to-use format

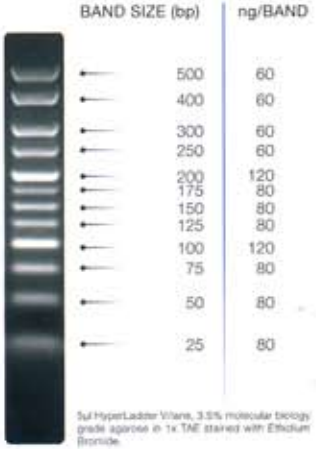
Description

A popular DNA Ladder with loading buffer in a single tube, enabling accurate sizing and quantitation of DNA between 25bp and 500bp. Each band contains an exact amount of DNA: 5µl per lane gives a total of 960ng of DNA. Bands at 100bp and 200bp have the highest intensity for easy identification and orientation. This ready-to-use format reduces handling steps and saves time; simply transfer HyperLadder V from the vial to the gel.

Storage Conditions

HyperLadder V should be stored at -20°C until first use, and thereafter at +4°C for up to 6 months. Avoid multiple freeze/thaw cycles.

HyperLadder V



BAND SIZE (bp)	ng/BAND
500	60
400	60
300	60
250	60
200	120
175	80
150	80
125	80
100	120
75	80
50	80
25	80

5µl HyperLadder V/lane, 3.5% molecular biology grade agarose in 1x TAE stained with Ethidium Bromide.

Associated Products

PRODUCT	CAT NO.	CHAPTER
Agarose Tablets	BIO-41028	Reagents for Molecular Biology
Coloured DNA Loading Buffers	BIO-37070	Reagents for Molecular Biology

HyperLadder VI

PRESENTATION	PACK SIZE	CAT NO.
2 x 1ml	200 Lanes	BIO-33033
5 x 5ml	500 Lanes	BIO-33034

HyperLadder VI includes: 5x Sample Loading Buffer

Size and Quantify DNA fragments from 10090bp to 48500bp

Features

- 10 bands (10090bp - 48500bp)
- Accurate quantitation
- Easy identification and orientation
- Ready-to-use format

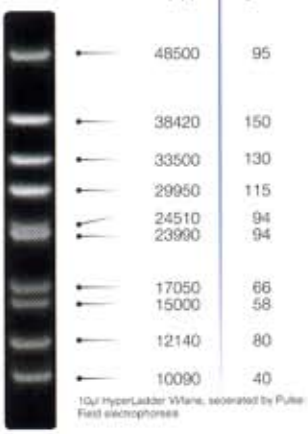
Description

A popular DNA Ladder with loading buffer in a single tube, enabling accurate sizing and quantitation of DNA between 10090bp and 48500bp. For superior resolution of fragments 29950bp to 48500bp, the use of Pulse Field Inversion Electrophoresis is recommended. HyperLadder VI also contains an additional corresponding band at 1500bp. Each band contains an exact amount of DNA: 10µl per lane gives a total of 830ng of DNA. A band at 38420bp has the highest intensity for easy identification and orientation. This ready-to-use format reduces handling steps and saves time; simply transfer HyperLadder VI from the vial to the gel.

Storage Conditions

HyperLadder VI should be stored at -20°C until first use, and thereafter at +4°C for up to 6 months. Avoid multiple freeze/thaw cycles.

HyperLadder VI



BAND SIZE (bp)	ng/BAND
48500	95
38420	150
33500	130
29950	115
24510	94
23990	94
17050	66
15000	58
12140	80
10090	40

10µl HyperLadder VI/lane, separated by Pulse Field electrophoresis

Associated Products

PRODUCT	CAT NO.	CHAPTER
Agarose Tablets	BIO-41028	Reagents for Molecular Biology
Coloured DNA Loading Buffers	BIO-37070	Reagents for Molecular Biology

EasyLadder I, II

PRODUCT	PRESENTATION	PACK SIZE	CAT NO.
EasyLadder I	2 x 500µl	200 Lanes	BIO-33045
EasyLadder I	5 x 500µl	500 Lanes	BIO-33046
EasyLadder II	2 x 500µl	200 Lanes	BIO-33047
EasyLadder II	5 x 500µl	500 Lanes	BIO-33048

EasyLadder I & II Include: 5x Sample Loading Buffer

Size and Quantify DNA fragments with Speed

Features

- Ideal for Short Runs
- 5 bands
- Accurate quantitation
- Ready-to-use format

Description

EasyLadders are designed for short runs (1 to 3cm) in standard or high-throughput agarose gels. EasyLadders contain only 5 bands each for easy identification of sample DNA bands during such short runs, and are supplied with red dye to indicate migration distance. Each band contains 50ng of DNA (5µl per lane gives a total of 250ng of DNA) enabling accurate sizing and quantitation of DNA samples.

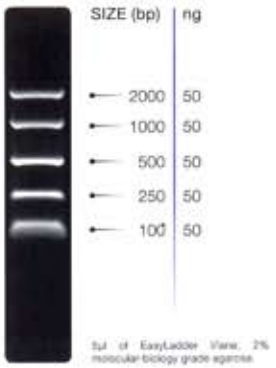
EasyLadder I can be used to size and quantify DNA fragments from 100bp to 2000bp, and EasyLadder II for DNA fragments from 500bp to 5000bp.

The ready-to-use format reduces handling steps and saves time; simply transfer EasyLadder from the vial to the gel.

Storage Conditions

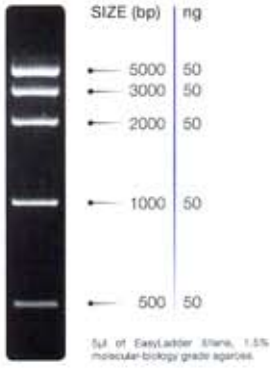
EasyLadders should be stored at -20°C until first use and thereafter at +4°C for up to 6 months. Avoid multiple freeze/thaw cycles.

EasyLadder I



5µl of EasyLadder I/lane, 2% molecular biology grade agarose.

EasyLadder II



5µl of EasyLadder II/lane, 1.5% molecular biology grade agarose.

Red Dye Migration

AGAROSE	RED DYE
0.7%	1500bp
1.0%	750bp
1.5%	500bp
2.0%	250bp
3.0%	75bp

Associated Products

PRODUCT	CAT NO.	CHAPTER
Agarose Tablets	BIO-41028	Reagents for Molecular Biology
Agarose	BIO-41026	Reagents for Molecular Biology
Coloured DNA Loading Buffers	BIO-37070	Reagents for Molecular Biology

2Kb MegaLadder

PRESENTATION	PACK SIZE	CAT NO.
1 x 100µl	200 Lanes	BIO-33035
4 x 100µl	500 Lanes	BIO-33036

2Kb MegaLadder Includes: 6x Sample Loading Buffer

Size High Molecular Weight DNA fragments from 2Kb to 80Kb

Features

- 40 bands in 2Kb increments (2Kb – 80Kb)
- Easy identification and orientation
- Separate sample loading buffer included
- Agarose-free PFI electrophoresis

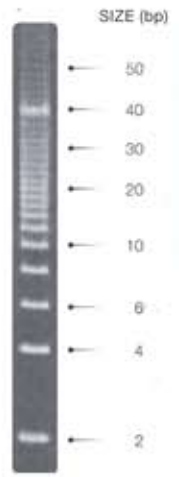
Description

A high molecular weight DNA Ladder, enabling the accurate sizing of DNA between 2Kb and 80Kb. A band at 40Kb has the highest intensity for easy identification and orientation. For the superior resolution of fragments above 30Kb, we recommend Pulse Field Inversion Electrophoresis.

Storage Conditions

2Kb MegaLadder can be stored for 12 months at -20°C.

2Kb MegaLadder



0.5µg of 2Kb Ladder, 0.5 x TBE, 1% agarose, gel length 18cm. Forward pulse 11v/cm, 1 sec; Reverse pulse 1.5v/cm, 1 sec. The separation of migration illustrated was achieved after 12 hours.

Associated Products

PRODUCT	CAT NO.	CHAPTER
Agarose Tablets	BIO-41028	Reagents for Molecular Biology
Agarose	BIO-41026	Reagents for Molecular Biology

5Kb MegaLadder

PRESENTATION	PACK SIZE	CAT NO.
1 x 100µl	200 Lanes	BIO-33037
4 x 100µl	500 Lanes	BIO-33038

5Kb MegaLadder Includes: 6x Sample Loading Buffer

Size High Molecular Weight DNA fragments from 5Kb to 150Kb

Features

- 30 bands in 5Kb increments (5Kb - 150Kb)
- Easy identification and orientation
- Separate sample loading buffer included
- Agarose-free PFI electrophoresis

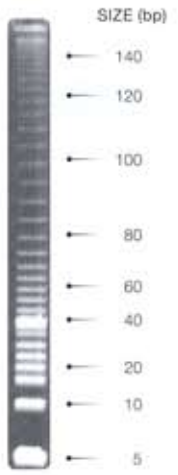
Description

A high molecular weight DNA Ladder, enabling the accurate sizing of DNA between 5Kb and 150Kb. A band at 40Kb has the highest intensity for easy identification and orientation. For the superior resolution of fragments above 30Kb, the use of Pulse Field Inversion Electrophoresis is recommended.

Storage Conditions

5Kb MegaLadder can be stored for 12 months at -20°C.

5Kb MegaLadder



1.5µg of 5kb DNA Ladder, 0.5 x TBE, 1% agarose, gel length 18cm. Forward pulse 11v/cm, 2 sec; Reverse pulse 1.5v/cm, 6 sec. The separation of migration illustrated here was achieved after 20 hours.

Associated Products

PRODUCT	CAT NO.	CHAPTER
Agarose Tablets	BIO-41028	Reagents for Molecular Biology
Agarose	BIO-41026	Reagents for Molecular Biology

RiboLadder Short

PRESENTATION	PACK SIZE	CAT NO.
1 x 25 Lanes	25 Lanes	BIO-33060

Size and Quantify RNA fragments from 100b to 1000b

Features

- Band sizes from 100b - 1000b
- Easy identification and orientation
- Loading Buffer included

Description

RiboLadder Short is a single-stranded RNA molecular weight marker with band sizes ranging from 100b to 1000b. A band at 500b has the highest intensity for easy identification and orientation. The ladder sequences are derived from transcribed lambda sequences using *in vitro* transcription. RiboLadder Short contains 20% formamide.

2x RNA Loading Buffer (with ethidium bromide) is supplied for loading RiboLadder and also samples. The inclusion of ethidium bromide makes it unnecessary to add this to the gel. If the RNA is to be used in a Northern Blot, we recommend using 2x RNA Loading Buffer without ethidium bromide (Cat No.BIO-37083).

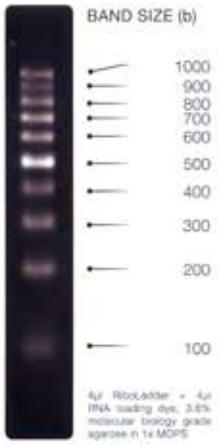
RNA Concentration

The RNA concentration in RiboLadder Short is 4.5µg/µl. For optimum resolution we recommend 4µl per lane (18µg/lane).

Storage Conditions

RiboLadder Short can be stored for 6 months at -70°C. Avoid multiple freeze/thaw cycles.

RiboLadder Short



Associated Products

PRODUCT	CAT NO.	CHAPTER
BioScript	BIO-27036	RNA Analysis
cDNA Synthesis Kit	BIO-65026	RNA Analysis
RNase Inhibitor	BIO-65028	RNA Analysis

RiboLadder Long

PRESENTATION	PACK SIZE	CAT NO.
1 x 25 Lanes	25 Lanes	BIO-33061

Size and Quantify RNA fragments from 500b to 9000b

Features

- Band sizes from 500b - 9000b
- Easy identification and orientation
- Loading Buffer included

Description

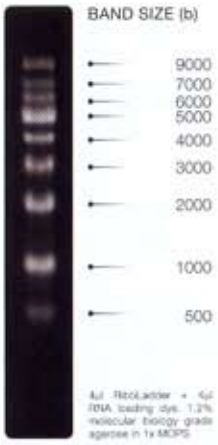
RiboLadder Long is a single-stranded RNA molecular weight marker with band sizes ranging from 500b to 9000b. A band at 5000b has the highest intensity for easy identification and orientation. The ladder sequences are derived from transcribed lambda sequences using *in vitro* transcription. RiboLadder Long contains 20% formamide.

2x RNA Loading Buffer (with ethidium bromide) is supplied for loading RiboLadder and also samples. The inclusion of ethidium bromide makes it unnecessary to add this to the gel. If the RNA is to be used in a Northern Blot, we recommend using 2x RNA Loading Buffer without ethidium bromide (Cat No.BIO-37083).

RNA Concentration

The RNA concentration in RiboLadder Long is 1.5µg/µl. For optimum resolution we recommend 4µl per lane (6µg/lane).

RiboLadder Long



Associated Products

PRODUCT	CAT NO.	CHAPTER
BioScript	BIO-27036	RNA Analysis
cDNA Synthesis Kit	BIO-65026	RNA Analysis
RNase Inhibitor	BIO-65028	RNA Analysis

Introduction to DHPLC Markers

Good practice in DHPLC analysis involves frequent validation of the resolving power of the column in any given instrument configuration. The analysis of DNA fragments under non-denaturing conditions provides a facile means of carrying out such diagnostic tests, thereby bringing a greater level of confidence to the analysis of sequence variation by DHPLC.

The pre-digested and purified pUC18 *Hae* III fragments provide an economical solution for monitoring column performance during DHPLC, which is especially important in high-throughput procedures. Note: *Hae* III produces blunt ends and therefore eliminates any interference that may arise from unpaired ends of the DNA duplexes during chromatography.

One of the principle ways of assessing the resolving power of a column is from its ability to resolve the 257 and 267 base pair fragments generated in the digestion of pUC18. In addition, the ability to separate DNA fragments over the range 11 - 587bp with a flat baseline, offers reassurance that the separation system is performing optimally.

DH-Ladder consists of three double-peaks, permitting an accurate determination of the resolving power of the system across a range of fragment sizes. It is ideally suited to high-throughput, multi-template, multi-sized DNA fragment analysis. The peaks 95, 105, 255, 270, 440 and 470bp DNA fragments are blunt-ended to eliminate interference from unpaired ends.

pUC18 *Hae* III digest

PACK SIZE	CAT NO.
200 injections	BIO-22028

Features

- Excellent control sample
- 11 DNA fragments (11bp - 587bp)
- 2 double-peaks for testing resolution
- Ready-to-use format

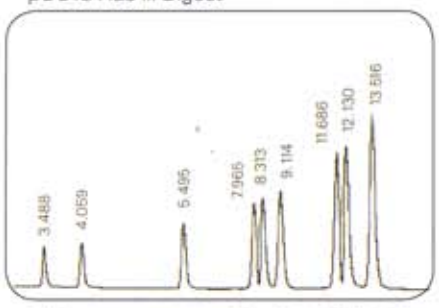
Description

pUC18 *Hae* III digest consists of pre-digested, highly purified fragments, and is designed for monitoring column performance during DHPLC. Before and during each run it is essential to determine the resolution of the column under non-denaturing conditions (50°C). A typical control run would include a range of fragments, with several DNA products of a similar size. The DNA fragments are: 11, 18, 80, 102, 174, 257, 267, 298, 434, 458 and 587bp.

Storage Conditions

pUC18 *Hae* III digest can be stored for 12months at -20°C.

pUC18 Hae III Digest



6µl Injection, separated on Varian Helix DHPLC with DNA Sep (Transgenomic) column.

Associated Products

PRODUCT	CAT NO.	CHAPTER
DH-Ladder	BIO-22029	Molecular Weight Markers

DH-Ladder

PACK SIZE	CAT NO.
200 injections	BIO-22029

Features

- Excellent control sample
- 3 double-peaks for testing resolution
- Fragments from 95bp - 470bp
- Ready-to-use format
- Ideally suited to high-throughput, multi-template, multi-sized DNA fragment analysis

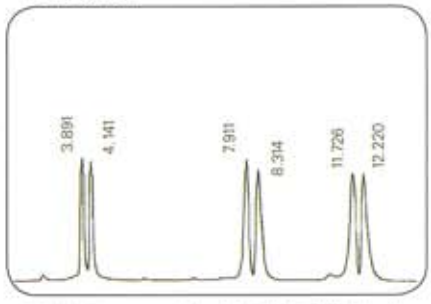
Description

DH-Ladder consists of 6 peaks arranged in 3 pairs, allowing resolution to be determined for small, medium and large fragments. DH-Ladder is designed for running pre, during and post-DHPLC, to determine if the sensitivity is uniform throughout the multi-sample run being analysed. The peaks are 95, 105, 255, 270, 440 and 470bp.

Storage Conditions

DH-Ladder can be stored for 12 months at -20°C.

DH-Ladder



6µl Injection, separated on Varian Helix DHPLC with DNA Sep (Transgenomic) column.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Mix	BIO-39028	Nucleotides
dNTP Set	BIO-39025	Nucleotides

Coloured DNA Loading Buffers

PRODUCT	PACK SIZE	CAT NO.
DNA Loading Buffer, Blue	2 x 1ml	BIO-37045
DNA Loading Buffer, Red	2 x 1ml	BIO-37068

Applications

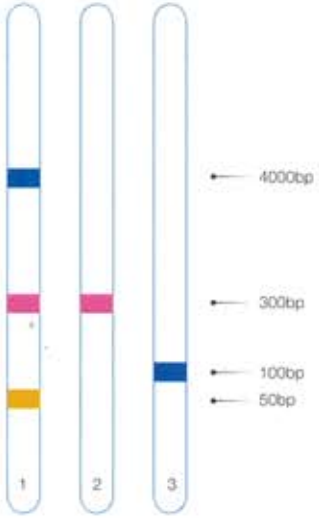
- To monitor migration rate during agarose electrophoresis

Description

5x Coloured DNA Loading Buffers

Associated Products

PRODUCT	CAT NO.	CHAPTER
Agarose Tablets	BIO-41028	Reagents for Molecular Biology
HyperLadder I	BIO-33025	Molecular Weight Markers
BIOTAQ Red	BIO-21041	Enzymes for Molecular Biology
dNTP Set	BIO-39025	Nucleotides



Graphic representation of Coloured DNA Loading Buffers in 2% agarose gel

Lane1: Tri-Colour DNA Loading Buffer
Lane 2: DNA Loading Buffer, Red
Lane 3: DNA Loading Buffer, Blue

Tri-Colour DNA Loading Buffer

PACK SIZE	CAT NO.
2 x 1ml	BIO-37070

Applications

- To monitor migration rate during agarose electrophoresis
- Three visible bands

Description

5x Tri-Colour DNA loading buffer.

Associated Products

PRODUCT	CAT NO.	CHAPTER
HyperLadder I	BIO-33025	Molecular Weight Markers
EasyLadder I	BIO-33046	Molecular Weight Markers

RNA Loading Buffers

PRODUCT	CONC.	PACK SIZE	CAT NO.
RNA Loading Buffer with Ethidium Bromide	2x	1ml	BIO-38025
RNA Loading Buffer without Ethidium Bromide	2x	1ml	BIO-38026

Features

- Available with or without Ethidium Bromide
- Ready-to-use solution

Applications

- Northern Blot analysis
- Agarose gel electrophoresis

Description

The new Bioline 2x RNA Loading Buffers maintain the denatured state of the RNA during electrophoresis. The ready-to-use buffer solutions are available either with or without ethidium bromide. RNA Loading Buffer with ethidium bromide includes ethidium bromide in the buffer, therefore it is not necessary to add it to the gel.

If the RNA is to be used in a Northern Blot, we recommend using RNA Loading Buffer without ethidium bromide. Ethidium bromide reduces hybridisation efficiency once the RNA is transferred to a membrane.

We recommend using RNA Loading Buffer on MOPS agarose gels. The buffer can also be used on formaldehyde, glyoxal, agarose gels and acrylamide urea gels.

Concentration

2x

Storage Conditions

RNA Loading Buffers can be stored for 12 months at -20°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
RiboLadder Short	BIO-33060	Molecular Weight Markers
RiboLadder Long	BIO-33061	Molecular Weight Markers
Agarose	BIO-41025	Reagents for Molecular Biology

FlipFlop Site-Directed Mutagenesis Kit

PACK SIZE	CAT NO.
30 Reactions	BIO-86030

Introduction

Site-directed mutagenesis is an invaluable process used in various areas of modern biology. It allows the determination of structural and functional characteristics of biomolecules. Various methods for this technique have been published, but they suffer from several disadvantages as they are generally time-consuming, laborious and/or require specially prepared template DNA.

The FlipFlop Site-Directed Mutagenesis Kit is a newly developed, highly efficient and fast working system for the generation of oligonucleotide-directed mutations and is designed to carry out site-directed mutagenesis in virtually any double-stranded plasmid of up to 12Kb. The Kit does not require any special treatment of the template, the entire mutagenesis reaction is completed within 15 thermal cycles and the protocol is simple and easy to use. Once mutagenesis is complete, the mutagenised plasmids are ready for transformation.

Features

- More than 90% efficiency
- Rapid, single tube protocol
- No cloning steps involved
- Optimised high-fidelity polymerase
- Involves a unique mechanism using HMdCTP
- Optimised for use with most common plasmids in the range 2.5Kb - 12Kb
- Hydroxymethylated dNTP mix
- Very efficient removal of background
- Includes easy-to-use control based on blue/white selection

Applications

- Generates up to 10 point mutations by the use of one single oligonucleotide
- Deletion or insertion of amino acids

Description

The FlipFlop Site-Directed Mutagenesis Kit contains all the necessary components to perform 30 mutagenesis reactions. It is optimised for the exchange of 1 to 10 nucleotides in one round of mutagenesis. The kit utilises a basic procedure that begins with a double-stranded plasmid with the insert of interest, and synthetic oligonucleotide primers containing the desired mutation. The generation of mutated plasmid is based on linear DNA amplification and is carried out using a special DNA polymerase mix, and a unique nucleotide mix where dCTP is replaced by hydroxymethylated-dCTP. Control plasmid and primers are provided.

The FlipFlop Site-Directed Mutagenesis Kit contains enough reagents to perform 30 mutagenesis reactions.

Kit components

- ACCUZYME DNA Polymerase
- FlipFlop Restriction Enzyme Mix
- 10x AccuBuffer
- 10x Hydroxymethylated dNTP Mix
- Oligonucleotide Control Primer #1
- Oligonucleotide Control Primer #2
- pUC19-MB Control Plasmid

Once mutagenesis is complete, the mutagenised plasmids can be used directly for transformation into competent cells. Note that competent cells are not included with the FlipFlop Site-Directed Mutagenesis Kit. Please refer to Chapter 4 Cloning for the full range of Bioline competent cells. When selecting competent cells it is essential to use *E.coli* cells with a *mcr*A$^-$, *mr*BC$^-$, or *mrr* genotype, such as Bioline CH3-Blue Chemically Competent Cells (Cat No.BIO-85040).

Storage Conditions

FlipFlop Site-Directed Mutagenesis Kit components should be stored at -20°C.

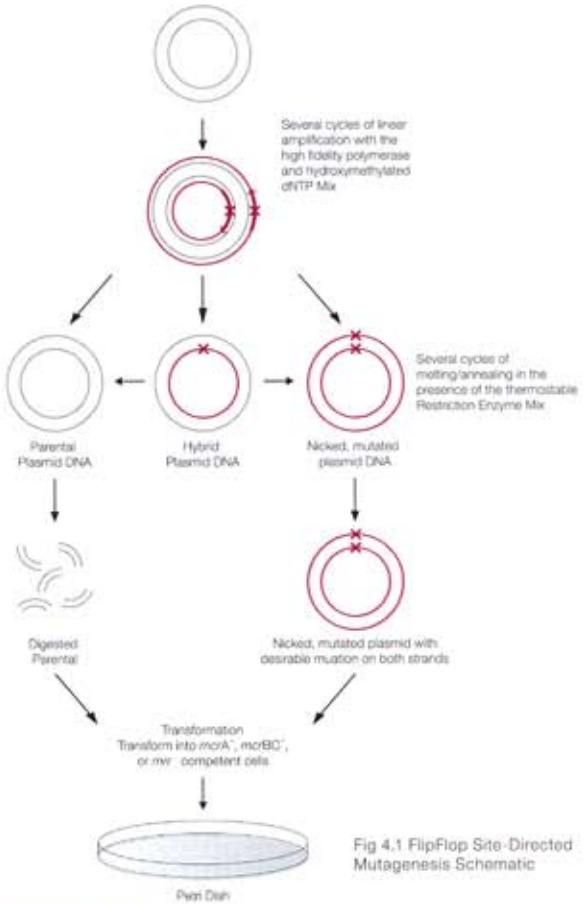


Fig 4.1 FlipFlop Site-Directed Mutagenesis Schematic

Associated Products

PRODUCT	CAT NO.	CHAPTER
CH3-Blue	BIO-85040	Cloning
Antibiotic Solutions	BIO-87025	Cloning
X-GAL/IPTG Solution	BIO-37086	Reagents for Molecular Biology
Thermo DNA Ligase	BIO-27045	Cloning
IPTG	BIO-37036	Reagents for Molecular Biology

Thermo DNA Ligase

PACK SIZE	CAT NO.
500 Units	BIO-27045
Includes: 10x Reaction Buffer	

Features

- Highly thermostable
- Ligates double-stranded DNA
- High specificity with broad range of DNA concentrations
- rATP dependent

Applications

- DNA ligations at high temperatures
- Ligase chain reactions (LCR)
- Detecting rolling circle mutation

Description

Thermo DNA Ligase is a recombinant enzyme that catalyses the joining of adjacent 5'-phosphate and 3'-hydroxy ends of double-stranded DNA at 45°C to 80°C. Thermo DNA Ligase is highly thermostable, with a half-life of over 60 minutes at 95°C, and is designed to provide higher ligation specificity and lower background than *Tth* DNA Ligase.

Concentration

10u/µl

Storage Conditions

Thermo DNA Ligase can be stored for 12 months at -20°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
18.2MΩ water	BIO-37080	Reagents for Molecular Biology
T4 DNA Ligase	BIO-27026	Cloning
IPTG	BIO-37036	Reagents for Molecular Biology
Quick-Stick Ligase	BIO-27027	Cloning
X-GAL	BIO-37035	Reagents for Molecular Biology

T4 DNA Ligase

PACK SIZE	CAT NO.
500 Units	BIO-27026
Includes: 10x T4 Ligase Buffer and ATP Solution	

Features

- Catalyses the joining of double-stranded DNA
- Exhibits high activity

Applications

- Ligation of cohesive and blunt-ended DNA fragments for cloning
- Sealing nicks in double-stranded DNA
- Ligation of synthetic linkers to blunt-ended DNA

Description

T4 DNA Ligase catalyses the joining of two strands of DNA between the 5'-phosphate and 3'-hydroxyl groups of adjacent nucleotides in either a blunt-ended or cohesive-ended configuration. T4 DNA Ligase catalyses the joining of RNA to either DNA or RNA strands in a duplex molecule, but will not join single-stranded nucleic acids.

Concentration

10u/µl

Storage Conditions

T4 DNA Ligase can be stored for 12 months at -20°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
Quick-Stick Ligase	BIO-27027	Cloning
Agarose	BIO-41025	Reagents for Molecular Biology
X-GAL/IPTG Solution	BIO-37086	Cloning

Quick-Stick Ligase

PACK SIZE	CAT NO.
50 Reactions	BIO-27027
100 Reactions	BIO-27028

Includes: 4x Quick-Stick Buffer

Features

- Rapid ligations of cohesive and blunt-ended DNA fragments
- Dramatically decreases the time required for DNA cloning
- No loss of transformation efficiency

Applications

- Cloning of DNA from: PCR fragments, plasmids, cosmids, genomic, phage and viral DNA
- Linker ligation
- Re-ligation of linearised plasmids
- Ligation of double-stranded oligonucleotides into vectors (plasmid and phage)

Description

Quick-Stick Ligase is designed to carry out fast and efficient ligation of both cohesive and blunt-ended DNA at room temperature. Quick-Stick Ligase is a T4 DNA Ligase that has been mutated to improve enzyme activity, and contains a specially developed 4x Quick-Stick buffer. The enzyme catalyses the joining of two strands of DNA between the 5'-phosphate and the 3'-hydroxyl groups of adjacent nucleotides in either a blunt-ended or cohesive-ended configuration.

Quick-Stick Ligase will ligate 99% of λ/*Hind*III cohesive-ended fragments, or 80% of λ/*EcoRV* blunt-ended fragments, in 5 minutes at room temperature. 100% ligation of blunt-ended fragments can be achieved by increasing the ligation time to 15 minutes at room temperature.

Concentration

10u/µl

Storage Conditions

Quick-Stick Ligase can be stored for 6 months at -20°C.

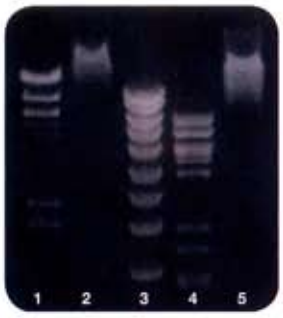


Fig 4.2 Ligation of cohesive or blunt-ended fragments with Bioline Quick-Stick Ligase. Lambda DNA was 10x over-digested with *EcorV* or *Hind* III, followed by heat inactivation. DNA fragments were ligated using the Bioline protocol for 5 minutes at room temperature.

Lane 1: *Hind* III-digested Lambda DNA (Cohesive ends)
Lane 2: *Hind* III-digested Lambda DNA ligated with Quick-Stick Ligase
Lane 3: HyperLadder I
Lane 4: *EcorV*-digested Lambda DNA (Blunt ends)
Lane 5: *EcorV*-digested Lambda DNA ligated with Quick-Stick Ligase

Uracil DNA Glycosylase (UDG)

PACK SIZE	CAT NO.
500 Units	BIO-27044

Features

- Recombinant source
- DNase/RNase-free
- Supplied with 10x Reaction Buffer

Application

- Removal of Uracil from Uracil-containing DNA

Description

Uracil DNA Glycosylase (UDG) catalyses the release of uracil from uracil-containing single-stranded or double-stranded DNA, but not from RNA.

Unit Definition

One unit is the amount of enzyme that catalyses the release of 60µmol of Uracil per minute from Uracil-containing double-stranded DNA.

Concentration

1u/µl

Storage Conditions

Uracil DNA Glycosylase can be stored for 6 months at -20°C.

Note: Purchase of this product does not convey a licence to perform any patented process.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dUTP	BIO-39035	Nucleotides

T4 Polynucleotide Kinase

PACK SIZE	CONC.	CAT NO.
500 Units Includes: 10x PNK Reaction Buffer	5u/µl	BIO-27031

Features

- Catalysis of γ-phosphate transfer from ATP to nucleic acids

Applications

- 5' Labelling of oligonucleotides for use as probes and sequencing primers
- 5' Labelling of single-stranded DNA and RNA molecules for use as probes
- Sequencing of DNA following protein footprinting
- End-labelling of DNA and RNA molecules for sequence determination

Description

T4 Polynucleotide Kinase is purified from recombinant *E. coli* strain JM109 which overproduces the enzyme. T4 Polynucleotide Kinase catalyses the transfer of the γ-phosphate from ATP to the 5'-terminus of polynucleotides or to mononucleotides bearing a 3'-phosphate group. T4 Polynucleotide Kinase is widely used to end-label short oligonucleotide probes and both DNA and RNA molecules for sequence determination.

Storage Conditions

T4 Polynucleotide Kinase can be stored for 12 months at -20°C.

IPTG

PRODUCT	PACK SIZE	CAT NO.
IPTG	5g	BIO-37036
IPTG Solution	10ml	BIO-37082
IPTG Solution	5 x 10ml	BIO-37083

Features

- Induces *E.coli lac* operon activity
- >99.6% by HPLC
- Available as powder and stabilised stock solution

Applications

- Blue/White colony screening
- Induction of *lac* operon for protein expression

Description

Isopropyl-ß-D-thiogalactopyranoside (IPTG) is a chemical analogue of lactose. Genes controlled by the *lac* or *tac* promoter/operator sequences are expressed to high levels in the presence of IPTG. IPTG is available as a powder or as a 1M stock solution (240mg/ml). We recommend using IPTG Solution at a concentration of between 0.1mM and 1mM.

Storage Conditions

IPTG can be stored for 12 months at -20°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
X-GAL	BIO-37035	Cloning
X-GAL/IPTG	BIO-37086	Cloning
Thermo DNA Ligase	BIO-27045	Cloning
Quick-Stick Ligase	BIO-27027	Cloning

X-GAL

PACK SIZE	CAT NO.
1g	BIO-37035

Features

- Extremely pure, 99.5% by TLC
- Intense blue precipitate upon hydrolysis

Applications

- Blue/White cloning systems
- Immunoblotting
- Immunocyto chemical assays
- Microbiology and cell culture media

Description

5-bromo-4-chloro-3-indolyl ß-D-galactopyranoside (X-GAL) is a chromogenic substrate for ß-Galactosidase that forms an intense blue precipitate. It can be used in Molecular Biology to detect the gal gene product, and also in Microbiology where it is used to detect micro-organisms which have β-Galactosidase activity (usually coliforms). It can be combined with the R-substrates to differentiate between two species of organisms on the same plate. X-GAL is soluble in N, N-dimethylformamide.

Storage Conditions

X-GAL can be stored for 12 months at -20°C. Store protected from light.

X-GAL/IPTG Solution

PACK SIZE	CAT NO.
10ml	BIO-37086

Features

- Convenient ready-to-use solution
- Ideal for Blue/White colony screening
- Compatible with all commonly used *lacZ* expression systems

Description

X-GAL/IPTG Solution is a ready-to-use solution of 40mg/ml X-GAL (5-bromo-4-chloro-3-indolyl ß-D-galactopyranoside) and 32mg/ml IPTG (Isopropyl-ß-D-1-thiogalactopyranoside), in a non-hazardous and non-toxic solvent (N-methyl-pyrrolidon). We recommend using X-GAL/IPTG Solution at a 1000x concentration. The X-GAL/IPTG Solution saves time and money, since no extra sterilisation is necessary, and it can be used directly from the freezer. The solution provides optimal blue/white colony screening and is compatible with all commonly used *lacZ* expression systems in bacteria, yeast and mammals.

Storage Conditions

X-GAL/IPTG Solution can be stored for 12 months at -20°C. Store protected from light.

Associated Products

PRODUCT	CAT NO.	CHAPTER
X-GAL	BIO-37035	Reagents for Molecular Biology
IPTG	BIO-37036	Reagents for Molecular Biology
Thermo DNA Ligase	BIO-27045	Cloning
Quick-Stick Ligase	BIO-27027	Cloning
T4 DNA Ligase	BIO-27026	Cloning

Antibiotic Solutions

PRODUCT	PACK SIZE	CONC.	CAT NO.
Ampicillin	10ml	100mg/ml	BIO-87025
Carbenicillin	10ml	100mg/ml	BIO-87026
Chloramphenicol	10ml	50mg/ml	BIO-87027
Kanamycin	10ml	50mg/ml	BIO-87028
Neomycin	10ml	50mg/ml	BIO-87029
Tetracycline	10ml	12.5mg/ml	BIO-87030

Features

- Cost effective
- Ready-to-use solutions
- Time saving
- Stable at -20°C
- Solutions in ethanol can be used directly from the freezer
- Avoid work with toxic or harmful substances
- No sterile filtration required

Antibiotic Properties

Antibiotics	Mode of Action	Mechanism of Resistance	Working Conc.	Stock Solution
Ampicillin	Ampicillin is a derivative of penicillin that causes cell death by interfering with bacterial cell wall synthesis.	Ampicillin resistance is mediated by cleavage of the β-lactam ring by β-lactamase (*bla* gene).	50-200µg/ml	100mg/ml in water
Carbenicillin	Carbenicillin is an ampicillin analogue that inhibits bacterial cell wall synthesis, and is commonly used in place of ampicillin to reduce the production of satellite colonies. Carbenicillin is more stable than ampicillin.	Carbenicillin resistance is mediated by cleavage of the β-lactam ring by β-lactamase (*bla* gene).	20-200µg/ml	100mg/ml in 50% ethanol
Kanamycin sulfate	Kanamycin sulfate causes cell death by binding to 70S ribosomal subunits, which inhibits ribosomal translocation and causes miscoding.	Kanamycin resistance is mediated by aminoglycoside phosphotransferase (*kan* gene), which inactivates kanamycin by phosphorylation.	10-50µg/ml	50mg/ml in water
Chloramphenicol	Chloramphenicol is a bacteriostatic agent that inhibits translation on the 50S ribosomal subunit, preventing peptide bond formation.	Chloramphenicol resistance is mediated by acetyltransferase (*cat* gene), which inactivates chloramphenicol by acetylation.	25-170µg/ml	50mg/ml in 100% ethanol
Tetracycline hydrochloride	Tetracycline inhibits protein synthesis by preventing binding of aminoacyl-tRNA to the 30S ribosomal subunit.	Tetracycline resistance is mediated by a protein (*tet* gene), which modifies the bacterial membrane and prevents transport of tetracycline into the cell.	12.5-50µg/ml	12.5mg/ml in 90% ethanol
Neomycin	Neomycin causes cell death by blocking protein synthesis. High concentrations of neomycin can cause toxicity in eukaryotic cells by interacting with mitochondrial ribosomes, and with reduced affinity, other eukaryotic ribosomes.	Neomycin resistance is mediated by aminoglycoside phosphotransferase (*nptII* gene), which inactivates neomycin by phosphorylation.	50µg/ml	50mg/ml in water

For Research Only

Associated Products

PRODUCT	CAT NO.	CHAPTER
α-Select Competent Cells	BIO-85027	Cloning
ElectroSHOX	BIO-85038	Cloning
X-GAL/IPTG Solution	BIO-37086	Cloning
BioBlue	BIO-85037	Cloning
FlipFlop Site-Directed Mutagenesis Kit	BIO-86030	Cloning

α-Select Competent Cells

PRODUCT	EFFICIENCY	PACK SIZE	CAT NO.
α-Select Bronze Efficiency	≥1 X 10^7 cfu/µg pUC19	2ml (10 x 200µl)	BIO-85025
α-Select Silver Efficiency	≥1 X 10^8 cfu/µg pUC19	2ml (10 x 200µl)	BIO-85026
α-Select Gold Efficiency	≥1 X 10^9 cfu/µg pUC19	1ml (20 x 50µl)	BIO-85027
α-Select Electrocompetent	≥1 X 10^9 cfu/µg pUC19	1ml (10 x 100µl)	BIO-85028

Features

- Comparable to DH5α™ *
- Variety of efficiencies: ≥10^7, ≥10^8, or ≥10^9 cfu/µg of pUC19
- Blue/White colour screening
- Accommodates larger plasmids
- Chemically Competent or Electroporation Grade

Applications

- Transformation of cloned DNA into bacterial cells
- Ideal for subcloning and generating cDNA libraries

Description

α-Select Competent Cells contain a *lacZ* marker that provides α-complementation of the β-galactosidase gene for blue/white colour screening. The cells are ideal for generating cDNA libraries and subcloning. α-Select Competent Cells also provide *rec*A1 and *end*A1 markers to minimize recombination and enhance the quality of the plasmid DNA. pUC19 DNA is also provided as a positive control.

Genotype

*deo*R *end*A1 *rec*A1 *rel*A1 *gyr*A96 *hsd*R17($r_k^- m_k^+$) *sup*E44 *thi*-1 Δ(*lac*ZYA-*arg*FV169) ϕ80δ*lac*ZΔM15 F^- γ$^-$

α-Select Competent Cells are available in a range of transformation efficiencies:

Bronze Efficiency ≥10^7 cfu/µg of pUC19
Silver Efficiency ≥10^8 cfu/µg of pUC19
Gold Efficiency ≥10^9 cfu/µg of pUC19 in convenient 50µl aliquots
Electrocompetent ≥10^9 cfu/µg of pUC19

Storage Conditions

α-Select Competent Cells should be stored at -70°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
T4 DNA Ligase	BIO-27026	Cloning
Quick-Stick Ligase	BIO-27027	Cloning
IPTG	BIO-37036	Reagents for Molecular Biology
X-GAL	BIO-37035	Reagents for Molecular Biology
T4 DNA Polymerase	BIO-27034	Enzymes for Molecular Biology

CH3-Blue Chemically Competent Cells

EFFICIENCY	PACK SIZE	CAT NO.
≥1 X 10^8 cfu/µg pUC19	1ml (10 x 100µl)	BIO-85039
≥1 X 10+ cfu/µg pUC19	1ml (20 x 50µl)	BIO-85040

Features

- Lacks *mcr*A, *mcr*BC, *mrr* and *hsd*RMS restriction systems
- Blue/White colour screening
- Available in two efficiencies: ≥10^8 or ≥10^9 cfu/µg of pUC19
- ≥10^9 efficiency cells provided in single-use 50µl aliquots
- Ideal for FlipFlop Site-Directed Mutagenesis Kit

Applications

- Cloning of methylated DNA
- Ideal for subcloning and generating cDNA libraries

Description

CH3-Blue Chemically Competent Cells are highly efficient *E. coli*, ideal for the construction of cDNA libraries using plasmid derived vectors. To facilitate the cloning of DNA that contains methylcytosine or 5-hydroxymethylcytosine, CH3-Blue lacks the *E. coli* restriction systems mcrA, mcrBC, mrr and *hsd*RMS. The *lacZ* mutation allows blue/white colour screening and α-complementation of recombinants. The *rec*A1 and *end*A1 markers minimise recombination events and improve the quality and yield of plasmid DNA.

Genotype

F^- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lacZ* ΔM15 Δ*lac*X74 *rec*A1 *end*A1 *ara* Δ139 Δ(*ara, leu*)7697 *galU galK* λ$^-$ *rps*L (Str^R) *nup*G

Storage Conditions

CH3-Blue Chemically Competent Cells should be stored at -80°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
FlipFlop Site-Directed Mutagenesis Kit	BIO-86030	Cloning
T4 DNA Ligase	BIO-27026	Cloning
Quick-Stick Ligase	BIO-27027	Cloning
IPTG	BIO-37036	Reagents for Molecular Biology
X-GAL	BIO-37035	Reagents for Molecular Biology
T4 DNA Polymerase	BIO-27034	Enzymes for Molecular Biology

* DH5α is a trademark of Invitrogen

ElectroSHOX Competent Cells

EFFICIENCY	PACK SIZE	CAT NO.
≥1 X 10^{10} cfu/µg pUC19	1ml (10 x 100µl)	BIO-85038

Features

- Comparable to DH10B™ *
- Blue/White colour screening
- *rec*A1 and *end*A1 markers improve plasmid DNA quality
- Lacks *E. coli* K restriction-modification system, to facilitate cloning of methylated genomic DNA
- Transformation of large plasmids (>30Kb)
- Highest efficiency available, producing ≥10^{10} cfu/µg of pUC19

Applications

- Construction of cDNA and Genomic DNA libraries
- Ideal for transformation of large plasmids (>30Kb)

ElectroSHOX Competent Cells are highly efficient *E. coli*, ideal for the construction of cDNA or genomic libraries using electroporation. The *lac*Z mutation allows blue/white colour screening and α-complementation of recombinants. The *rec*A1 and *end*A1 markers minimise recombination events and improve the quality and yield of plasmid DNA. In order to facilitate cloning of methylated genomic DNA, ElectroSHOX lacks *E. coli* K restriction-modification systems, and is ideal for the transformation of large plasmids (>30Kb).

Genotype

F⁻ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lacZ* ΔM15 Δ*lac*X74 *rec*A1 *end*A1 *ara* Δ139 Δ(*ara, leu*)7697 *gal*U *gal*K λ- *rps*L (Str^R) *nup*G

Storage Conditions

ElectroSHOX Competent Cells should be stored at -80°C.

*DH10B is a trademark of Invitrogen

Associated Products

PRODUCT	CAT NO.	CHAPTER
T4 DNA Ligase	BIO-27026	Cloning
Quick-Stick Ligase	BIO-27027	Cloning
IPTG	BIO-37036	Reagents for Molecular Biology
X-GAL	BIO-37035	Reagents for Molecular Biology
T4 DNA Polymerase	BIO-27034	Enzymes for Molecular Biology

BIO*Blue* Chemically Competent Cells

EFFICIENCY	PACK SIZE	CAT NO.
≥1 X 10^8 cfu/µg pUC19	1ml (10 x 100µl)	BIO-85036
≥1 X 10^9 cfu/µg pUC19	1ml (20 x 50µl)	BIO-85037

Features

- Blue/White colour screening
- Single-stranded plasmid rescue
- Antibiotic resistance facilitated F' episome maintenance
- Two efficiencies: ≥10^8 or ≥10^9 cfu/µg of DNA

Applications

- Ideal strain for preparation of high-quality plasmid DNA

Description

BIO*Blue* Chemically Competent Cells provide an ideal host for optimal preparation of both high-quality plasmid and lambda phage vectors. The BIO*Blue* strain allows blue/white colour screening through α-complementation of the β-Galactosidase gene. The *end*A1 phenotype allows production of high-quality plasmid DNA. Single-stranded DNA can be produced from plasmids containing a phage f1 origin. BIO*Blue* is also an excellent host for M13 and related filamentous phage, and supports blue/white plaque screening and phage production. Maintenance of the F' episome in this strain is facilitated via selection with tetracycline, unlike strains such as JM101 which require growth on minimal media. This strain is available in efficiencies of both >10^8 and >10^9 cfu/µg of pUC19.

Genotype

*rec*A1 *end*A1 *gyr*A96 *thi*-1 *hsd*R17($r_k^- m_k^+$) *sup*E44 *rel*A1 *lac* [F' *pro*AB *lacI*^{q}ZΔM15 Tn10(Tet^r)]

Storage Conditions

BIO*Blue* Chemically Competent Cells should be stored at -70°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
T4 DNA Ligase	BIO-27026	Cloning
Quick-Stick Ligase	BIO-27027	Cloning
IPTG	BIO-37036	Reagents for Molecular Biology
X-GAL	BIO-37035	Reagents for Molecular Biology
T4 DNA Polymerase	BIO-27034	Enzymes for Molecular Biology

BL21 Competent Cells

PRODUCT	EFFICIENCY	PACK SIZE	CAT NO.
BL21	≥ 1 X 10^7cfu/µg	1ml (10 x 100µl)	BIO-85031
BL21 (DE3)	≥ 1 X 10^7cfu/µg	1ml (10 x 100µl)	BIO-85032
BL21 (DE3) pLysS	≥ 1 X 10^7cfu/µg	1ml (10 x 100µl)	BIO-85033
BL21 (DE3) pLysE	≥ 1 X 10^7cfu/µg	1ml (10 x 100µl)	BIO-85034
BL21 Combo Pack	≥ 1 X 10^7cfu/µg	1.5ml (15 x 100µl)	BIO-85035

Features

- **High-Level Protein Expression**
- **Protease deficient**
- **Provided in convenient 100µl aliquots**
- **Transformation Efficiency: ≥1 x 10^7 cfu/µg of pUC19**

Description

BL21 and its λDE3 lysogenic derivatives are all-purpose *E. coli* host strains for high-level expression of a variety of recombinant proteins. All strains are deficient in both *lon* and *ompT* proteases, resulting in a higher level of intact recombinant proteins. BL21 competent cells are an ideal host for optimal expression of proteins from vectors utilizing *E. coli* promoters (this strain lacks a source of T7 RNA polymerase).

The BL21(DE3) competent cells are designed for high-level protein expression and easy induction using T7 promoter constructs. These strains are lysogens of bacteriophage DE3, a lambda derivative containing the gene for T7 RNA polymerase under control of the *lacUV5* promoter. Induction with IPTG allows production of T7 RNA Polymerase, which then directs the expression of the target gene located downstream of the T7 promoter in the expression vector. Each BL21 (DE3) strain provides varying degrees of regulation and expression control. Recombinant proteins that are non-toxic to *E. coli* are generally expressed at higher levels in BL21 (DE3) cells than in BL21 (DE3) pLysS or BL21 (DE3) pLysE.

BL21:

Ideal host for protein expression from vectors containing *E. coli* promoters.
Genotype: F⁻ *ompT* $hsdS_B(r_B^-m_B^-)$ *gal dcm*

BL21 (DE3):

General purpose host for T7 vector protein expression.
Genotype: F⁻ *ompT* $hsdS_B(r_B^-m_B^-)$ *gal dcm* (DE3)

BL21 (DE3) pLysS:

Carries plasmid pLysS that constitutively expresses T7 lysozyme, a natural inhibitor of T7 RNA Polymerase. This strain is used to minimize basal level expression of potentially toxic gene products before induction with IPTG.
Genotype: F⁻ *ompT* $hsdSB(r_B^-m_B^-)$ *gal dcm* (DE3) pLysS (Cam^R)

BL21 (DE3) pLysE:

Carries plasmid pLysE that expresses a higher level of T7 lysozyme by virtue of the tet promoter. This plasmid provides a higher level of repression of the T7 RNA Polymerase gene prior to induction.
Genotype: F⁻ *ompT* $hsdSB(r_B^-m_B^-)$ *gal dcm* (DE3) pLysE (Cam^R)

Storage Conditions

BL21 Competent Cells should be stored at -70°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
T4 DNA Ligase	BIO-27026	Cloning
Quick-Stick Ligase	BIO-27027	Cloning
IPTG	BIO-37036	Reagents for Molecular Biology
X-GAL	BIO-37035	Reagents for Molecular Biology
T4 DNA Polymerase	BIO-27034	Enzymes for Molecular Biology

BioScript

CONC.	PACK SIZE	CAT NO.
200u/µl	10000 Units	BIO-27036

Ultra-Stable Reverse Transcriptase

Features

- Ultra-stable: No loss of activity detected after 1 week at room temperature
- Ultra-pure (DNase/RNase-free)
- High yields (low RNase H activity)
- Highly sensitive
- Optimal temperature range 37°C - 50°C
- The high-quality cDNA obtained is suitable for real-time PCR experiments

Applications

- First strand cDNA synthesis
- Production of templates for RT-PCR
- cDNA library construction

Description

BioScript is a Moloney Murine Leukaemia Virus (MMLV) Reverse Transcriptase, which exhibits high stability and is active at a wide range of temperatures. Unlike the wild-type enzyme, BioScript possesses low RNase H activity, which results in enhanced yields. In addition, BioScript is highly sensitive even when the amount of template is a limiting factor.

BioScript is suitable for first strand cDNA synthesis, cDNA library construction, and the production of templates for RT-PCR analysis of gene expression. BioScript can be used with total RNA, mRNA or *in vitro* transcribed RNA.

Storage Conditions

BioScript can be stored for 6 months at -20°C.

No RNase Activity

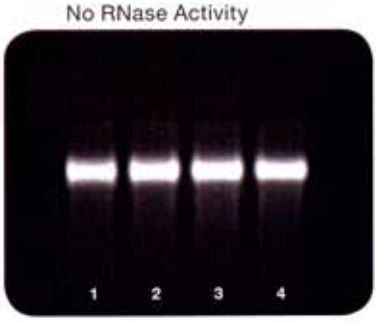


Fig 5.1 2µg of 1.5Kb poly(A)-tailed RNA were incubated with 200u of BioScript at 37°C for one hour, followed by phenol extraction and then subjected to formaldehyde gel analysis

Lane 1: RNA fragment with RNase-free water kept on ice for one hour
Lane 2: RNA fragment with RNase-free water incubated at 37°C for one hour
Lane 3: RNA fragment in BioScript reaction buffer incubated at 37°C for one hour
Lane 4: RNA fragment in BioScript reaction buffer incubated at 37°C for one hour in the presence of 200u of BioScript

Ultra-stable - 1 Week Stability Assay

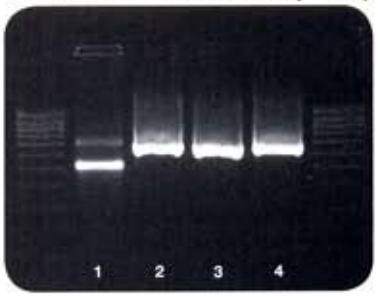


Fig 5.2 Reverse Transcription experiments using an *in vitro* RNA transcript and Bioscript. A 500bp fragment was amplified using both Bioscript and its corresponding buffer stored at the following temperatures (lanes 2-4) for one week

Lane 1: Control-no reverse transcriptase
Lane 2: -20°C
Lane 3: +4°C
Lane 4: Room temperature

Note: lanes 2, 3 and 4 show a band corresponding to the RNA: DNA hybrid, as opposed to lane 1 where only RNA is observed

Associated Products

PRODUCT	CAT NO.	CHAPTER
BIOTAQ	BIO-21040	Enzymes for Molecular Biology
IMMOLASE	BIO-21047	Enzymes for Molecular Biology
ACCUZYME	BIO-21052	Enzymes for Molecular Biology
BioMix	BIO-25012	Enzymes for Molecular Biology
dNTP Set	BIO-39025	Nucleotides
RiboLadder Short	BIO-33060	Molecular Weight Markers
RiboLadder Long	BIO-33061	Molecular Weight Markers

cDNA Synthesis Kit

CONC.	PACK SIZE	CAT NO.
200u/µl	30 Reactions	BIO-65025
200u/µl	100 Reactions	BIO-65026

Features

- Enough reagents to perform 100 single-strand reactions
- Convenient, reliable, cost-effective
- The high-quality cDNA obtained is suitable for real-time PCR experiments
- cDNA suitable for library construction and RT-PCR experiments

Description

The Bioline cDNA Synthesis Kit contains all the necessary components to generate cDNA from an RNA template. The kit is based on our ultra-stable MMLV reverse transcriptase (BioScript). Unlike the wild-type enzyme, BioScript possesses low RNase H activity and is suitable for first strand cDNA synthesis, cDNA library construction, and the production of templates for RT-PCR amplifications.

The cDNA Synthesis Kit contains enough reagents to perform 100 single-strand reactions.
cDNA Synthesis Kit components:

- BioScript
- 5x BioScript Reaction Buffer
- DEPC-treated Water
- Ultra-pure dNTPs
- Control RNA Template
- RNase Inhibitor
- Oligo $(dT)_{18}$ Primer (for poly A mRNA templates)
- Random Hexamer Primers

Storage Conditions

The cDNA Synthesis Kit can be stored for 6 months at -20°C. BioScript will remain stable if stored as specified.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Set	BIO-39025	Nucleotides
RiboLadder Short	BIO-33060	Molecular Weight Markers
RiboLadder Long	BIO-33061	Molecular Weight Markers
Agarose	BIO-41025	Reagents for Molecular Biology

High Sensitivity

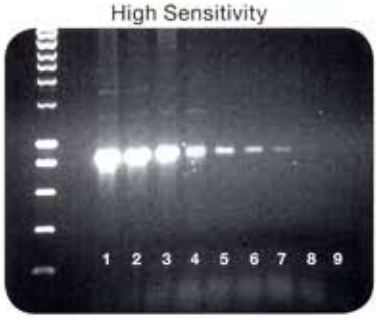


Fig 5.3 Serial template dilution experiment: Various quantities (as indicated) of total HeLa cell RNA were reverse-transcribed using BioScript and oligo$(dT)_{18}$ primer in a 20µl reaction, i.e.: (1) 50ng, (2) 25ng, (3) 10ng, (4) 1ng, (5) 500pg, (6) 250pg, (7) 100pg, (8) 50pg and (9) 0pg. Subsequently, 5µl of each reaction were used in conjunction with β-Actin primer to amplify an 860bp band from human mRNA.

Active at a wide range of temperatures

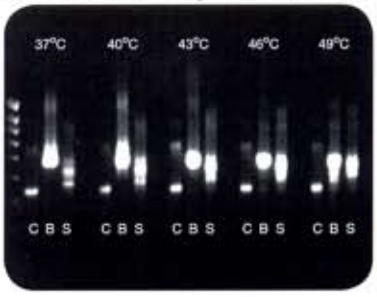


Fig 5.4 Reverse Transcription experiments using an *in vitro* RNA transcript in conjunction with either:

Lane C: no RT/control
Lane B: BioScript
Lane S: competitor SIII
at various temperatures.

T7 RNA Polymerase

CONC.	PACK SIZE	CAT NO.
20u/µl	5000 Units	BIO-21055
200u/µl	20000 Units	BIO-21056

Features

- High level of purity, specificity and activity
- Readily incorporates NTPs

Applications

- *in vitro* transcription
- Generating labelled RNA probes
- Producing RNA of defined length

Description

T7 RNA Polymerase is a DNA-dependent RNA polymerase, which catalyses RNA synthesis in the 5'-3' direction downstream from any DNA with a T7 promoter sequence. T7 RNA Polymerase can be used to produce RNA from cloned inserts and will incorporate labelled NTPs. Products of this enzyme can be used as probes for screening purposes.

Also included is the Bioline 2x Ultimate Reaction Buffer, a proprietary buffer specially formulated and optimised to give exceptional performance and reliability.

T7 Promoter Sequence

The binding and transcription initiation of T7 RNA Polymerase is dependent upon the presence of a T7 promoter sequence:

5' TAATACGACTCACTATAGGGAGA 3'

The red sequence represents the minimum promoter sequence required for efficient transcription, with the blue G being the first base incorporated into the resulting RNA. The final 4 bases (GAGA) are not necessary, but many templates will show enhanced efficiency and yield if those extra bases are present.

Double-Stranded Transcription

T7 RNA Polymerase can be used to make double-stranded RNA for restriction into siRNA. To achieve this, both the forward and reverse primer must contain the T7 promoter sequence. The resulting transcripts can be annealed to each other by heating to 65°C for 5 minutes and cooling slowly.

Storage Conditions

T7 RNA Polymerase can be stored for 12 months at -20°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
NTP Mix	BIO-39050	Nucleotides
T7 Transcription Kit	BIO-21072	RNA Analysis
BioScript	BIO-27036	RNA Analysis
RNase Inhibitor	BIO-65027	RNA Analysis

T7 Transcription Kit

CONC.	PACK SIZE	CAT NO.
20u/µl	50 Reactions	BIO-21072

Features

- Enough reagents to perform up to 1ml of high yield RNA transcription
- Convenient, reliable, cost-effective

Description

The T7 Transcription Kit contains all the necessary components to carry out fast and efficient transcription of RNA from any DNA with a T7 promoter sequence, and can be used to produce RNA from cloned inserts. The enzyme will incorporate labelled NTPs, and products of this enzyme can be used as probes for screening purposes.

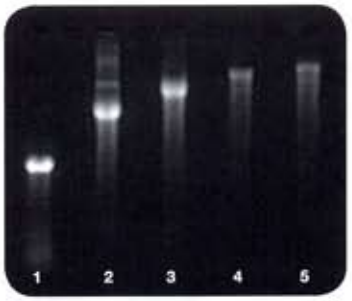
The T7 Transcription Kit contains enough reagents to perform either 50 x 20µl of high yield RNA transcription, or 1 x 1ml.

T7 Transcription Kit components:

- Highly-purified recombinant T7 RNA polymerase
- 2x Ultimate Buffer (specially formulated transcription buffer)
- DEPC-treated Water
- Ultra-pure NTPs
- Control Template
- RNase Inhibitor
- DTT

Storage Conditions

T7 RNA Polymerase can be stored for 12 months at -20°C. DTT should be aliquotted to avoid instability due to multiple freeze/thaw cycles.



PCR products of different lengths *in vitro* transcribed using the T7 Transcription Kit.
Lane 1: 1Kb RNA product
Lane 2: 3Kb RNA product
Lane 3: 5Kb RNA product
Lane 4: 7Kb RNA product
Lane 5: 9Kb RNA product

Associated Products

PRODUCT	CAT NO.	CHAPTER
T7 RNA Polymerase	BIO-21055	RNA Analysis
18.2MΩ Water	BIO-37080	Reagents for Molecular Biology
BioScript	BIO-27036	RNA Analysis

RiboLadder Short

PRESENTATION	PACK SIZE	CAT NO.
1 x 25 Lanes	25 Lanes	BIO-33060

Size and Quantify RNA fragments from 100b to 1000b

Features

- Band sizes from 100b - 1000b
- Easy identification and orientation
- Loading Buffer included

Description

RiboLadder Short is a single-stranded RNA molecular weight marker with band sizes ranging from 100b to 1000b. A band at 500b has the highest intensity for easy identification and orientation. The ladder sequences are derived from transcribed lambda sequences using *in vitro* transcription. RiboLadder Short contains 20% formamide.

2x RNA Loading Buffer (with ethidium bromide) is supplied for loading RiboLadder and also samples. The inclusion of ethidium bromide makes it unnecessary to add this to the gel. If the RNA is to be used in a Northern Blot, we recommend using 2x RNA Loading Buffer without ethidium bromide (Cat No.BIO-37083).

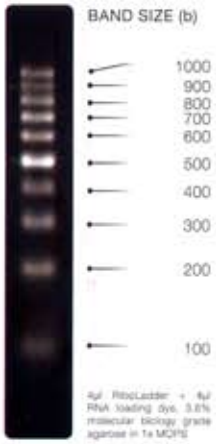
RNA Concentration

The RNA concentration in RiboLadder Short is 4.5µg/µl. For optimum resolution we recommend 4µl per lane (18µg/lane).

Storage Conditions

RiboLadder Short can be stored for 6 months at -70°C. Avoid multiple freeze/thaw cycles.

RiboLadder Short



Associated Products

PRODUCT	CAT NO.	CHAPTER
BioScript	BIO-27036	RNA Analysis
cDNA Synthesis Kit	BIO-65026	RNA Analysis
RNase Inhibitor	BIO-65028	RNA Analysis

RiboLadder Long

PRESENTATION	PACK SIZE	CAT NO.
1 x 25 Lanes	25 Lanes	BIO-33061

Size and Quantify RNA fragments from 500b to 9000b

Features

- Band sizes from 500b - 9000b
- Easy identification and orientation
- Loading Buffer included

Description

RiboLadder Long is a single-stranded RNA molecular weight marker with band sizes ranging from 500b to 9000b. A band at 5000b has the highest intensity for easy identification and orientation. The ladder sequences are derived from transcribed lambda sequences using *in vitro* transcription. RiboLadder Long contains 20% formamide.

2x RNA Loading Buffer (with ethidium bromide) is included for loading RiboLadder and for loading samples. Since ethidium bromide is included in the loading buffer, it is not necessary to add it to the gel. If the RNA is to be used in a northern blot, we recommend using 2x RNA Loading Buffer without ethidium bromide (Cat No. BIO-37083).

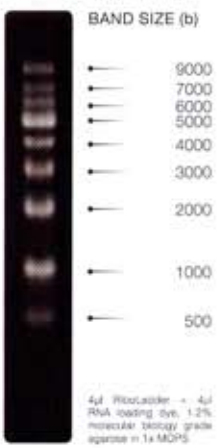
RNA Concentration

The RNA concentration in RiboLadder Long is 1.5µg/µl, for optimum resolution we recommend 4µl per lane (6µg/Lane).

Storage Conditions

RiboLadder Long can be stored for 6 months at -70°C. Avoid multiple freeze/thaw cycles.

RiboLadder Long



Associated Products

PRODUCT	CAT NO.	CHAPTER
BioScript	BIO-27036	RNA Analysis
cDNA Synthesis Kit	BIO-65026	RNA Analysis
RNase Inhibitor	BIO-65028	RNA Analysis

T4 RNA Ligase

CONC.	PACK SIZE	CAT NO.
20u/µl	1000 Units	BIO-65032

Features

- Recombinant source
- Broad range of substrates
- ATP-dependent

Applications

- Labelling 3'-end of RNA with 5'-[^{32}P] pCp
- Intra- and Intermolecular ligation of ssRNA and ssDNA
- mRNA 5'-end tagging with oligonucleotides for mapping or cDNA synthesis
- Synthesis of single-stranded oligo-deoxyribonucleotides

Description

T4 RNA Ligase is a recombinant enzyme that catalyses the joining of a 5' phosphoryl-terminated nucleic acid donor to a 3' hydroxyl-terminated nucleic acid acceptor via the formation of a phosphodiester bond. T4 RNA Ligase is active on single-stranded RNA and DNA, oligo-ribonucleotides, oligo-deoxyribonucleotides, dinucleoside pyrophosphates and many nucleotide derivatives.

Concentration

20u/µl

Storage Conditions

T4 RNA Ligase should be stored at -20°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
T7 RNA Polymerase	BIO-21055	RNA Analysis
RNA Loading Buffer	BIO-38025	Reagents for Molecular Biology
RNase Inhibitor	BIO-65027	RNA Analysis
RiboLadder Short	BIO-33060	RNA Analysis

RNase Inhibitor

CONC.	PACK SIZE	CAT NO.
40u/µl	2500 Units	BIO-65027
40u/µl	10000 Units	BIO-65028

Features

- Highly effective
- Nuclease and Nickase-free
- Complete inhibition of RNAse A,B and C

Applications

- RNA purification
- cDNA preparation by reverse transcription for cloning and hybridisation
- RNA sequencing
- RT-PCR
- *in vitro* RNA transcription
- *in vitro* protein synthesis

Description

Ribonuclease Inhibitor (RNase Inhibitor) is a recombinant protein. It inhibits different RNases (A, B, C) by binding non-covalently in a 1:1 ratio, with an association constant of 10^{14}. RNase Inhibitor is useful in any applications where the presence of RNases is a potential problem. RNase Inhibitor is tested for activity, SDS-PAGE purity, and the absence of endonucleases, nickases and exonucleases.

Storage Conditions

RNase Inhibitor can be stored for 6 months at -20°C. Avoid multiple freeze/thaw cycles.

Associated Products

PRODUCT	CAT NO.	CHAPTER
BioScript	BIO-27036	RNA Analysis
T7 RNA Polymerase	BIO-21055	RNA Analysis
dNTP Set	BIO-39025	Nucleotides
RiboLadder Short	BIO-33060	Molecular Weight Markers
RiboLadder Long	BIO-33061	Molecular Weight Markers
Agarose	BIO-41025	Reagents for Molecular Biology

NTPs

PRODUCT	PACK SIZE	FINAL CONC.	PRESENTATION	CAT NO.
NTP Set	4 x 25µmol	100mM	4 x 250µl	BIO-39052
NTP Mix	100µmol	100mM	1ml	BIO-39050

Features

- Suitable for *in vitro* transcription
- >98% pure by HPLC
- Supplied as 100mM solutions
- Sets or a convenient mix

Applications

- *in vitro* transcription reactions
- Production of RNA probes and transcripts

Description

Bioline NTPs (Ribonucleoside-5'-tri-phosphates) are manufactured in-house and are tested in functional assays with the Bioline RNA polymerases. Bioline NTPs are >98% pure as analysed by HPLC and are free of DNase, RNase, Protease, Phosphatase and nicking activity.

The NTP Set consists of 4 separate 100mM solutions (ATP, GTP, CTP, and UTP, (pH 7.5)) as sodium salts. Each solution contains 25µmol (250µl) of the corresponding NTP. For *in vitro* RNA synthesis, mix equal volumes of all separate NTP solutions.

The NTP Mix is a solution containing 25µmol of each ATP, GTP, CTP and UTP (pH 7.5) as sodium salts in a convenient mix at 100mM (total NTP concentration).

Storage Conditions

NTPs can be stored for 12 months at -20°C. Avoid multiple freeze/thaw cycles. For long-term usage, aliquoting is recommended.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Set	BIO-39025	Nucleotides
dNTP Mix	BIO-39028	Nucleotides
Agarose	BIO-41025	Reagents for Molecular Biology
T7 RNA Polymerase	BIO-21055	RNA Analysis
T7 Transcription Kit	BIO-21072	RNA Analysis

TRIsure

PRESENTATION	PACK SIZE	CAT NO.
TRIsure	100ml	BIO-38032
TRIsure	200ml	BIO-38033

Features

- Quick, ready-to-use reagent for simultaneous isolation of RNA, DNA and protein
- Convenient 1hr protocol
- Performs well with large or small amounts of tissue or cells

Description

TRIsure is a ready-to-use reagent for the isolation of total RNA from cells and tissues. TRIsure maintains the integrity of the extracted RNA, while disrupting cells and subsequently dissolving cell components. After the addition of chloroform, the RNA can be recovered easily from the aqueous phase. DNA and protein can also be recovered after further precipitation steps. The RNA remains in the aqueous phase and is subsequently recovered by precipitation with isopropyl alcohol.

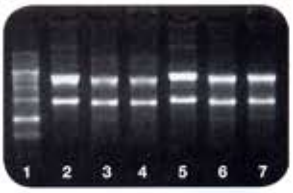
The isolated RNA is suitable for any downstream application such as RT-PCR, hybridisation assays, or *in vitro* transcription. 1ml of TRIsure is sufficient to isolate total RNA from 1 x 10^7 cells or 100mg of tissue.

From 1 x 10^6 of cultured cells, the expected yield of RNA is:

- 8-15µg from epithelial cells
- 5-7µg from fibroblasts

Storage Conditions

TRIsure should be stored at +4°C



RNA extracted from 3T6 cells and mouse tissue, using Bioline TRIsure and Competitor I.

Lane 1: RiboLadder Long
Lane 2: 4µg of total RNA from 3T6 cells - Competitor I
Lane 3: 4µg of total RNA from mouse kidney tissue -Competitor I
Lane 4: 4µg of total RNA from mouse liver tissue - Competitor I
Lane 5: 4µg of total RNA from 3T6 cells - Bioline TRIsure
Lane 6: 4µg of total RNA from mouse kidney tissue - Bioline TRIsure
Lane 7: 4µg of total RNA from mouse liver tissue - Bioline TRIsure

Associated Products

PRODUCT	CAT NO.	CHAPTER
BioScript	BIO-27036	RNA Analysis
RNA Loading Buffer	BIO-38025	Molecular Weight Markers
RiboLadder Short	BIO-33060	Molecular Weight Markers
RiboLadder Long	BIO-33061	Molecular Weight Markers

Agarose (DNase/RNase-free)

PACK SIZE	CAT NO.
100g	BIO-41026
500g	BIO-41025

Features

- Excellent value and clarity
- High gel strength (>1500g/cm²)
- DNase/RNase-free

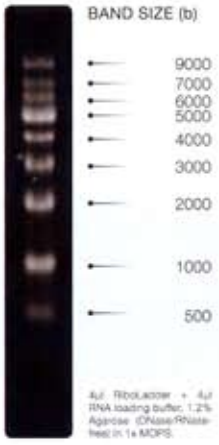
Applications

- DNA/RNA Electrophoresis
- Ideal for separating nucleic acids of a wide range of sizes, especially large fragments (>10Kb)

Description

Bioline Agarose (DNase/RNase-free) is an extremely pure, high molecular grade agarose powder that has been extensively tested for RNase contamination, and is suited to DNA and RNA electrophoresis. The high gel strength (>1500g/cm²) means that gels as low as 0.5% are practicable.

High Resolution Electrophoresis



4µl RiboLadder + 4µl RNA loading buffer, 1.2% Agarose (DNase/RNase-free) in 1x MOPS.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Set	BIO-39025	Nucleotides
2x PolyMate Additive	BIO-37041	Reagents for Molecular Biology
BIOTAQ	BIO-21060	Enzymes for Molecular Biology
RNA Loading Buffer	BIO-38025	Molecular Weight Markers

Oligo $(dT)_{18}$

PACK SIZE	CAT NO.
27µg	BIO-38029

Description

Oligo $(dT)_{18}$ is suitable for use as a primer for first strand cDNA synthesis with a reverse transcriptase. The primer hybridises to the poly-adenylated ends of mRNA templates.

5'-d (TTT TTT TTT TTT TTT TTT)-3'

Concentration

100µl at 270ng/µl

Directions

Use 1µl in a 20µl reverse transcription reaction

Storage Conditions

Oligo $(dT)_{18}$ can be stored for 12 months at -20°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
cDNA Synthesis Kit	BIO-65026	RNA Analysis
BioScript	BIO-27036	RNA Analysis
RiboLadder Long	BIO-33061	Molecular Weight Markers
DEPC-treated Water	BIO-38031	RNA Analysis

10x MOPS Buffer

COMPOSITION	PACK SIZE	CAT NO.
0.2M MOPS (pH 7), 20mM NaAc, 10mM EDTA (pH 8)	1 litre	BIO-38027

Description

MOPS (3-Morpholinopropanesulfonic acid)-EDTA-Sodium Acetate Buffer is especially designed for agarose gel electrophoresis of RNA. The buffer is supplied at a 10x concentration and is ready for use. For optimal denaturation and visualisation of samples we recommend Bioline RNA Loading Buffers (Cat No.BIO-38025 and BIO-38026).

Storage Conditions

10x MOPS Buffer can be stored for 3 months at room temperature. Avoid exposure to light and moisture.

Associated Products

PRODUCT	CAT NO.	CHAPTER
DEPC-treated Water	BIO-38030	RNA Analysis
RNA Loading Buffer with Ethidium Bromide	BIO-38025	Molecular Weight Markers
RNA Loading Buffer w/out Ethidium Bromide	BIO-38026	Molecular Weight Markers
Agarose	BIO-41025	Reagents for Molecular Biology

Random Hexamer Primers

PACK SIZE	CAT NO.
25µg	BIO-38028

Description

Random Hexamer Primers are commonly used for priming single-stranded DNA or RNA for extension by DNA polymerases or reverse transcriptases.

5' – d (NNNNNN) –3' N = G, A, T or C

Concentration

50µl at 50ng/µl

Directions

Use 1-5µl in a 20µl reverse transcription reaction

Storage Conditions

Random Hexamer Primers can be stored for 12 months at -20°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
BioScript	BIO-27036	RNA Analysis
cDNA Synthesis Kit	BIO-65026	RNA Analysis
BIOTAQ	BIO-21040	Enzymes for Molecular Biology

DEPC-treated Water

PACK SIZE	CAT NO.
10 x 10ml	BIO-38030
1 Litre	BIO-38031

Description

DEPC-treated Water is ideal for use in all RNA work. DEPC-treated Water is prepared by treating ultra-pure 18.2MΩ Water with diethylpyrocarbonate (DEPC), and is then autoclaved to inactivate the DEPC.

Storage Conditions

DEPC-treated Water can be stored for 12 months at -20°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
RiboLadder Short	BIO-33060	Molecular Weight Markers
10x MOPS Electrophoresis Buffer	BIO-38027	RNA Analysis
RNA Loading Buffer	BIO-38025	Molecular Weight Markers
BioScript	BIO-27036	RNA Analysis

Protein Tools

Bioline offers a wide range of *E.coli* host strains to facilitate high-efficiency cloning and gene expression. Rigorous quality control standards ensure lot-to-lot consistency and the highest transformation efficiencies possible. Our competent cells are available in a wide range of transformation efficiencies and in convenient packaging formats.

Several cloning strains are available which offer distinct genotype advantages for use in particular applications. These strains include desirable genetic markers, which allow for useful applications such as blue/white colour screening, cloning of methylated DNA and the transformation of large plasmids.

Bioline offers a series of BL21 Competent Cells for optimal protein expression and expression control. BL21 Competent Cells are suitable for high-level expression of a variety of recombinant proteins, and are ideal hosts for the expression of proteins from vectors utilizing *E.coli* promoters.

Bioline Competent Cell Selection Chart

APPLICATION	α-Select	CH3-BLUE	BIO*Blue*	Electro**SHOX**	BL21	BL21 (DE3)	BL21 (DE3) pLysS	BL21 (DE3) pLysE
Routine Subcloning	**	*	*	*				
cDNA Libraries	*	**	*	**				
Best Quality Plasmid DNA	*	*	**	*				
Large Plasmids	*			**				
Single Stranded Phage DNA		**	**					
Methylated cDNA		**		**				
Genomic DNA				**				
Non-T7 Promoter Protein Expression					**	*		
T7 Promoter Expression						**	*	*
Regulation of T7 Expression						*	*	***

* : Compatible ** : Recommended *** : Optimal

α-Select Competent Cells

PRODUCT	EFFICIENCY	PACK SIZE	CAT NO.
α-Select Bronze Efficiency	≥1 X 10^7 cfu/µg pUC19	2ml (10 x 200µl)	BIO-85025
α-Select Silver Efficiency	≥1 X 10^8 cfu/µg pUC19	2ml (10 x 200µl)	BIO-85026
α-Select Gold Efficiency	≥1 X 10^9 cfu/µg pUC19	1ml (20 x 50µl)	BIO-85027
α-Select Electrocompetent	≥1 X 10^9 cfu/µg pUC19	1ml (10 x 100µl)	BIO-85028

Features

- Comparable to DH5α™ *
- Variety of efficiencies: ≥10^7, ≥10^8, or ≥10^9 cfu/µg of pUC19
- Blue/White colour screening
- Accommodates larger plasmids
- Chemically Competent or Electroporation Grade

Applications

- Transformation of cloned DNA into bacterial cells
- Ideal for subcloning and generating cDNA libraries

Description

α-Select Competent Cells contain a *lacZ* marker that provides α-complementation of the β-galactosidase gene for blue/white colour screening. The cells are ideal for generating cDNA libraries and subcloning. α-Select Competent Cells also provide *rec*A1 and *end*A1 markers to minimize recombination and enhance the quality of the plasmid DNA. pUC19 DNA is also provided as a positive control.

Genotype

*deo*R *end*A1 *rec*A1 *rel*A1 *gyr*A96 *hsd*R17($r_k^- m_k^+$) *sup*E44 *thi*-1 Δ(*lac*ZYA-*arg*FV169) ϕ80δ*lac*ZΔM15 F^- γ$^-$

α-Select Competent Cells are available in a range of transformation efficiencies:

Bronze Efficiency ≥10^7 cfu/µg of pUC19
Silver Efficiency ≥10^8 cfu/µg of pUC19
Gold Efficiency ≥10^9 cfu/µg of pUC19 in convenient 50µl aliquots
Electrocompetent ≥10^9 cfu/µg of pUC19

Storage Conditions

α-Select Competent Cells should be stored at -70°C.

* DH5α is a trademark of Invitrogen

Associated Products

PRODUCT	CAT NO.	CHAPTER
T4 DNA Ligase	BIO-27026	Cloning
Quick-Stick Ligase	BIO-27027	Cloning
IPTG	BIO-37036	Reagents for Molecular Biology
X-GAL	BIO-37035	Reagents for Molecular Biology
T4 DNA Polymerase	BIO-27034	Enzymes for Molecular Biology

CH3-Blue Chemically Competent Cells

EFFICIENCY	PACK SIZE	CAT NO.
≥1 X 10^8 cfu/µg pUC19	1ml (10 x 100µl)	BIO-85039
≥1 X 10+ cfu/µg pUC19	1ml (20 x 50µl)	BIO-85040

Features

- Lacks *mcr*A, *mcr*BC, *mrr* and *hsd*RMS restriction systems
- Blue/White colour screening
- Available in two efficiencies: ≥10^8 or ≥10^9 cfu/µg of pUC19
- ≥10^9 efficiency cells provided in single-use 50µl aliquots
- Ideal for FlipFlop Site-Directed Mutagenesis Kit

Applications

- Cloning of methylated DNA
- Ideal for subcloning and generating cDNA libraries

Description

CH3-Blue Chemically Competent Cells are highly efficient *E. coli*, ideal for the construction of cDNA libraries using plasmid derived vectors. To facilitate the cloning of DNA that contains methylcytosine or 5-hydroxymethylcytosine, CH3-Blue lacks the *E. coli* restriction systems mcrA, mcrBC, mrr and *hsd*RMS. The *lacZ* mutation allows blue/white colour screening and α-complementation of recombinants. The *rec*A1 and *end*A1 markers minimise recombination events and improve the quality and yield of plasmid DNA.

Genotype

F^- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lacZ* ΔM15 Δ*lac*X74 *rec*A1 *end*A1 *ara* Δ139 Δ(*ara, leu*)7697 *gal*U *gal*K λ$^-$ *rps*L (Str^R) *nup*G

Storage Conditions

CH3-Blue Chemically Competent Cells should be stored at -80°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
FlipFlop Site-Directed Mutagenesis Kit	BIO-86030	Cloning
T4 DNA Ligase	BIO-27026	Cloning
Quick-Stick Ligase	BIO-27027	Cloning
IPTG	BIO-37036	Reagents for Molecular Biology
X-GAL	BIO-37035	Reagents for Molecular Biology
T4 DNA Polymerase	BIO-27034	Enzymes for Molecular Biology

ElectroSHOX Competent Cells

EFFICIENCY	PACK SIZE	CAT NO.
≥1 X 10^{10} cfu/µg pUC19	1ml (10 x 100µl)	BIO-85038

Features

- Comparable to DH10B™ *
- Blue/White colour screening
- *rec*A1 and *end*A1 markers improve plasmid DNA quality
- Lacks *E. coli* K restriction-modification system, to facilitate cloning of methylated genomic DNA
- Transformation of large plasmids (>30Kb)
- Highest efficiency available, producing ≥10^{10} cfu/µg of pUC19

Applications

- Construction of cDNA and Genomic DNA libraries
- Ideal for transformation of large plasmids (>30Kb)

ElectroSHOX Competent Cells are highly efficient *E. coli*, ideal for the construction of cDNA or genomic libraries using electroporation. The *lacZ* mutation allows blue/white colour screening and α-complementation of recombinants. The *rec*A1 and *end*A1 markers minimise recombination events and improve the quality and yield of plasmid DNA. In order to facilitate cloning of methylated genomic DNA, ElectroSHOX lacks *E. coli* K restriction-modification systems, and is ideal for the transformation of large plasmids (>30Kb).

Genotype

F⁻ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lacZ* ΔM15 Δ*lacX*74 *rec*A1 *end*A1 *ara* Δ139 Δ(*ara, leu*)7697 *gal*U *gal*K λ- *rps*L (Str^R) *nup*G

Storage Conditions

ElectroSHOX Competent Cells should be stored at -80°C.

*DH10B is a trademark of Invitrogen

Associated Products

PRODUCT	CAT NO.	CHAPTER
T4 DNA Ligase	BIO-27026	Cloning
Quick-Stick Ligase	BIO-27027	Cloning
IPTG	BIO-37036	Reagents for Molecular Biology
X-GAL	BIO-37035	Reagents for Molecular Biology
T4 DNA Polymerase	BIO-27034	Enzymes for Molecular Biology

BIO*Blue* Chemically Competent Cells

EFFICIENCY	PACK SIZE	CAT NO.
≥1 X 10^{8} cfu/µg pUC19	1ml (10 x 100µl)	BIO-85036
≥1 X 10^{9} cfu/µg pUC19	1ml (20 x 50µl)	BIO-85037

Features

- Blue/White colour screening
- Single-stranded plasmid rescue
- Antibiotic resistance facilitated F' episome maintenance
- Two efficiencies: ≥10^{8} or ≥10^{9} cfu/µg of DNA

Applications

- Ideal strain for preparation of high-quality plasmid DNA

Description

BIO*Blue* Chemically Competent Cells provide an ideal host for optimal preparation of both high-quality plasmid and lambda phage vectors. The BIO*Blue* strain allows blue/white colour screening through α-complementation of the β-Galactosidase gene. The *end*A1 phenotype allows production of high-quality plasmid DNA. Single-stranded DNA can be produced from plasmids containing a phage f1 origin. BIO*Blue* is also an excellent host for M13 and related filamentous phage, and supports blue/white plaque screening and phage production. Maintenance of the F' episome in this strain is facilitated via selection with tetracycline, unlike strains such as JM101 which require growth on minimal media. This strain is available in efficiencies of both >10^{8} and >10^{9} cfu/µg of pUC19.

Genotype

*rec*A1 *end*A1 *gyr*A96 *thi*-1 *hsd*R17($r_k^- m_k^+$) *sup*E44 *rel*A1 *lac* [F' *pro*AB *lacI*^{q}ZΔM15 Tn10(Tet^r)]

Storage Conditions

BIO*Blue* Chemically Competent Cells should be stored at -70°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
T4 DNA Ligase	BIO-27026	Cloning
Quick-Stick Ligase	BIO-27027	Cloning
IPTG	BIO-37036	Reagents for Molecular Biology
X-GAL	BIO-37035	Reagents for Molecular Biology
T4 DNA Polymerase	BIO-27034	Enzymes for Molecular Biology

BL21 Competent Cells

PRODUCT	EFFICIENCY	PACK SIZE	CAT NO.
BL21	≥ 1 X 10^7cfu/µg	1ml (10 x 100µl)	BIO-85031
BL21 (DE3)	≥ 1 X 10^7cfu/µg	1ml (10 x 100µl)	BIO-85032
BL21 (DE3) pLysS	≥ 1 X 10^7cfu/µg	1ml (10 x 100µl)	BIO-85033
BL21 (DE3) pLysE	≥ 1 X 10^7cfu/µg	1ml (10 x 100µl)	BIO-85034
BL21 Combo Pack	≥ 1 X 10^7cfu/µg	1.5ml (15 x 100µl)	BIO-85035

Features

- **High-Level Protein Expression**
- **Protease deficient**
- **Provided in convenient 100µl aliquots**
- **Transformation Efficiency: ≥1 x 10^7 cfu/µg of pUC19**

Description

BL21 and its λDE3 lysogenic derivatives are all-purpose *E. coli* host strains for high-level expression of a variety of recombinant proteins. All strains are deficient in both *lon* and *ompT* proteases, resulting in a higher level of intact recombinant proteins. BL21 competent cells are an ideal host for optimal expression of proteins from vectors utilizing *E. coli* promoters (this strain lacks a source of T7 RNA polymerase).

The BL21(DE3) competent cells are designed for high-level protein expression and easy induction using T7 promoter constructs. These strains are lysogens of bacteriophage DE3, a lambda derivative containing the gene for T7 RNA polymerase under control of the *lacUV5* promoter. Induction with IPTG allows production of T7 RNA Polymerase, which then directs the expression of the target gene located downstream of the T7 promoter in the expression vector. Each BL21 (DE3) strain provides varying degrees of regulation and expression control. Recombinant proteins that are non-toxic to *E. coli* are generally expressed at higher levels in BL21 (DE3) cells than in BL21 (DE3) pLysS or BL21 (DE3) pLysE.

BL21:

Ideal host for protein expression from vectors containing *E. coli* promoters.
Genotype: F⁻ *ompT* $hsdS_B(r_B^- m_B^-)$ *gal dcm*

BL21 (DE3):

General purpose host for T7 vector protein expression.
Genotype: F⁻ *ompT* $hsdS_B(r_B^- m_B^-)$ *gal dcm* (DE3)

BL21 (DE3) pLysS:

Carries plasmid pLysS that constitutively expresses T7 lysozyme, a natural inhibitor of T7 RNA Polymerase. This strain is used to minimize basal level expression of potentially toxic gene products before induction with IPTG.
Genotype: F⁻ *ompT* $hsdSB(r_B^- m_B^-)$ *gal dcm* (DE3) pLysS (Cam^R)

BL21 (DE3) pLysE:

Carries plasmid pLysE that expresses a higher level of T7 lysozyme by virtue of the tet promoter. This plasmid provides a higher level of repression of the T7 RNA Polymerase gene prior to induction.
Genotype: F⁻ *ompT* $hsdSB(r_B^- m_B^-)$ *gal dcm* (DE3) pLysE (Cam^R)

Storage Conditions

BL21 Competent Cells should be stored at -70°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
T4 DNA Ligase	BIO-27026	Cloning
Quick-Stick Ligase	BIO-27027	Cloning
IPTG	BIO-37036	Reagents for Molecular Biology
X-GAL	BIO-37035	Reagents for Molecular Biology
T4 DNA Polymerase	BIO-27034	Enzymes for Molecular Biology

Metal Chelating Cellulose

PRESENTATION	CAT NO.
25ml	BIO-75029
50ml	BIO-75030

Features

- One-step purification from crude lysate to >95% pure protein
- High binding affinity and capacity
- Purification under native or denaturing conditions
- Precharged, ready-to-use matrices

Applications

- High-performance purification of recombinant proteins with: natural surface-exposed histidine residues, engineered histidine tags or histidine clusters
- Protein refolding (matrix-assisted refolding) evaluation of protein folding status
- Preparative group fractionation of complex extracts and biofluids

Immobilised Metal Ion Affinity Sorbent

Immobilised metal ion affinity chromatography (IMAC) utilises the interaction between chelated transition metal ions and the histidine side chains on proteins. Metal Chelating Cellulose is an IMAC medium consisting of beads of cellulose, to which a tetradentate chelating group has been attached covalently . As Ni^{2+} is the preferred metal ion for His-tagged protein purification, the attached chelating group may be charged with Ni^{2+}. Other transition metal ions like Co^{2+} or Cu^{2+} may be used.

Description

Metal Chelating Cellulose resin is a newly developed, highly efficient medium designed for high-performance purification of His-tagged recombinant proteins in one easy step. This medium has low metal ion leakage, high protein-binding capacity, excellent protein-binding stability, and is compatible with a broad range of additives used in protein purification.

The base matrix is Beaded Cellulose, which consists of non-cross-linked pure cellulose beads. It provides Chelating Cellulose resin with high physical stability and excellent flow characteristics. Tetradentate chelator is linked to the Cellulose matrix via an 11-atom hydrophilic spacer arm, which provides very stable binding and prevents steric hindrance problem.

Bulk Orders

Bioline is a primary manufacturer and can accommodate multi-litre orders for Metal Chelating Cellulose. Please contact your nearest Bioline representative or distributor for a quote.

Note: Certain applications of this product may be covered by patents in certain countries. Purchase of this product does not convey a licence to perform any patented process.

Characteristics

Matrix	Beaded Cellulose
Dynamic Binding Capacity	At least 30-40 mg His-tagged protein per ml of the medium
Bead Volume	15.7ml/g
Matrix Exclusion Limit	4 x 10^6 Da
Average Particle Size	80-100µm
Maximum Linear Flow Rate (H_2O)	450cm/h
Recommended Flow Rate	< 400cm/h
Maximum Operating Pressure (H_2O)	1.5-2.0 bar
Chemical Stability	Stable in: 0.01M HCl, 0.1M NaOH. Tested for 1 week at 40°C
	1M NaOH, 70% acetic acid. Tested for 12 hours
	2%SDS. Tested for 1 hour
	30% 2-propanol. Tested for 1 hour
pH Stability (Ni^{2+} - stripped medium)	Short Term (< 2 hours) 2-4
Long Term (< 1 week)	3-12 hrs
Storage	20% ethanol in water
Storage Temperature	+4°C

Glutathione Cellulose

PRESENTATION	CAT NO.
25ml	BIO-75027
50ml	BIO-75028

Features

- One-step purification from crude lysate to >95% pure protein
- High binding affinity and capacity
- Purification under native conditions
- Ready-to-use matrices

Applications

- High-performance purification of GST-tagged recombinant proteins

Description

Glutathione Cellulose is an affinity chromatography medium that offers rapid, mild and non-denaturing affinity purification of GST, glutathione peroxidase, glyoxalase I, and recombinant GST fusion proteins. GST-tagged proteins can be purified directly from pre-treated bacterial lysates using Glutathione Cellulose. The tagged proteins are eluted under mild, non-denaturing conditions that preserve protein antigenicity and function.

The base matrix is Beaded Cellulose, which consists of non-cross-linked pure cellulose beads. It provides Glutathione Cellulose with high physical stability and excellent flow characteristics. Glutathione is linked to the Cellulose matrix via an 11-atom hydrophilic linker, which provides a very stable binding, even in alkaline conditions.

Note

The binding of GST to Glutathione is dependent on flow rate, and lower flow rates often increase the binding capacity. This is important during sample loading and elution.

Bulk Orders

Bioline is a primary manufacturer and can accommodate multi-litre orders for Glutathione Cellulose. Please contact your nearest Bioline representative or distributor for a quote.

Characteristics

Matrix	Beaded Cellulose
Dynamic Binding Capacity	> 10mg GST-tagged protein per ml of medium
Ligand	Glutathione coupled via 11-atom linker arm
Ligand Concentration	10-20µmol of glutathione per ml of medium (based on NH_2 groups)
Bead Volume	15.7ml/g
Matrix Exclusion Limit	4 x 10^6 Da
Average Particle Size	80-100µm
Maximum Linear Flow Rate (H_2O)	450cm/h
Recommended Flow Rate	< 400cm/h
Maximum Operating Pressure (H_2O)	1.5-2.0 bar
Chemical Stability	All commonly used aqueous buffers e.g. 1M acetate (pH 4), 6M Guanidine HCl for 1 hour at room temperature
Short term pH Stability (< 2 hours)	2-14 hrs
Long term pH Stability (< 1 week)	4-10 hrs
Storage	20% ethanol in water
Storage Temperature	+4°C

Heparin Cellulose

PRESENTATION	CAT NO.
25ml	BIO-75025
50ml	BIO-75026

Features

- One-step purification from crude lysate to >95% pure protein
- High binding affinity and capacity
- Purification under native or denaturing conditions
- Ready-to-use matrices

Applications

High-performance purification of:

- Blood coagulation factors
- Lipoprotein lipases
- Reverse transcriptases
- DNA polymerases
- RNA polymerases
- Growth factors
- Restriction endonucleases

Description

Heparin is a highly sulfated glycosaminoglycan composed of repeating disaccharide units with anticoagulant properties. Immobilised heparin is used in the purification of blood coagulation factors and lipoprotein lipases. There are several growth factors that bind to heparin, these include: fibroplast growth factor, endothelial cell growth factor, and cartilage-derived growth factor. Recombinant HIV-1 reverse transcriptase can be purified to homogeneity by using immobilised heparin in combination with other methods of chromatography.

By coupling heparin to a Beaded Cellulose using a chemically optimised linkage, Heparin Cellulose provides an excellent medium for both laboratory and process scale affinity chromatography.

The base matrix is Beaded Cellulose, which consists of highly beaded non-cross-linked pure cellulose beads. It provides Heparin Cellulose with high physical stability and excellent flow characteristics. Heparin is linked to the Cellulose matrix by reductive amination, which provides a very stable binding, even in alkaline conditions.

Bulk Orders

Bioline is a primary manufacturer and can accommodate multi-litre orders for Heparin Cellulose. Please contact your nearest Bioline representative or distributor for a quote.

Characteristics

Matrix	Beaded Cellulose
Ligand	Heparin coupled via 10-carbon linker are
Ligand Concentration	1.5-2.0mg of heparin per ml of medium (colourmetric determination)
Bead Volume	15.7ml/g
Matrix Exclusion Limit	4×10^6 Da
Average Particle Size	80-100µm
Maximum Linear Flow Rate (H_2O)	450cm/h
Recommended Flow Rate	< 400cm/h
Maximum Operating Pressure (H_2O)	1.5-2.0 bar
Chemical Stability	All commonly used aqueous buffers e.g. 50mM acetate (pH 4), 6M Guanidine HCl, 8M Urea, 4M NaCl, 0.1M NaOH (1 week at room temp)
Short term pH Stability (< 2 hours)	2-14 hrs
Long term pH Stability (< 1 week)	3-12 hrs
Storage	20% ethanol in water
Storage Temperature	+4°C
Sanitisation	0.1M NaOH in 20% ethanol in water for 1 hour at room temp

Proteinase K

PRODUCT	PACK SIZE	CAT NO.
Proteinase K	100mg	BIO-37037
Proteinase K	1000mg	BIO-37038
Proteinase K Solution	5ml	BIO-37084
Proteinase K Solution	5 x 5ml	BIO-37085

Features

- Broad-spectrum serine protease
- Active under denaturing conditions
- Stable at high temperatures
- Molecular biology grade
- Available as powder and stabilised stock solution

Applications

- Inactivation of RNases/DNases during nucleic acid extraction
- Protein modification
- General protein digestion

Description

Proteinase K is an enzyme used to digest most proteins in molecular-biological techniques. The enzyme may be used at 56°C for up to 4 hours, or 37°C for overnight incubations. Proteinase K is available as a powder or as a stabilised stock solution (20mg/ml). Proteinase K solution is stabilised with a specially formulated buffer, and can be used directly from the freezer.

Storage Conditions

Proteinase K can be stored for 12 months at -20°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Set	BIO-39025	Nucleotides
dNTP Mix	BIO-39028	Nucleotides
2x PolyMate Additive	BIO-37041	Reagents for Molecular Biology
Hyper Ladder I	BIO-33025	Molecular Weight Markers

X-GAL

PACK SIZE	CAT NO.
1g	BIO-37035

Features

- Extremely pure, 99.5% by TLC
- Intense blue precipitate upon hydrolysis

Applications

- Blue/White cloning systems
- Immunoblotting
- Immunocyto chemical assays
- Microbiology and cell culture media

Description

5-bromo-4-chloro-3-indolyl ß-D-galactopyranoside (X-GAL) is a chromogenic substrate for ß-Galactosidase that forms an intense blue precipitate. It can be used in Molecular Biology to detect the gal gene product, and also in Microbiology where it is used to detect micro-organisms which have β-Galactosidase activity (usually coliforms). It can be combined with the R-substrates to differentiate between two species of organisms on the same plate. X-GAL is soluble in N, N-dimethylformamide.

Storage Conditions

X-GAL can be stored for 12 months at -20°C. Store protected from light.

Associated Products

PRODUCT	CAT NO.	CHAPTER
IPTG	BIO-37036	Cloning
X-GAL/IPTG	BIO-37086	Cloning
Thermo DNA Ligase	BIO-27045	Cloning
T4 DNA Ligase	BIO-27026	Cloning

SureClean

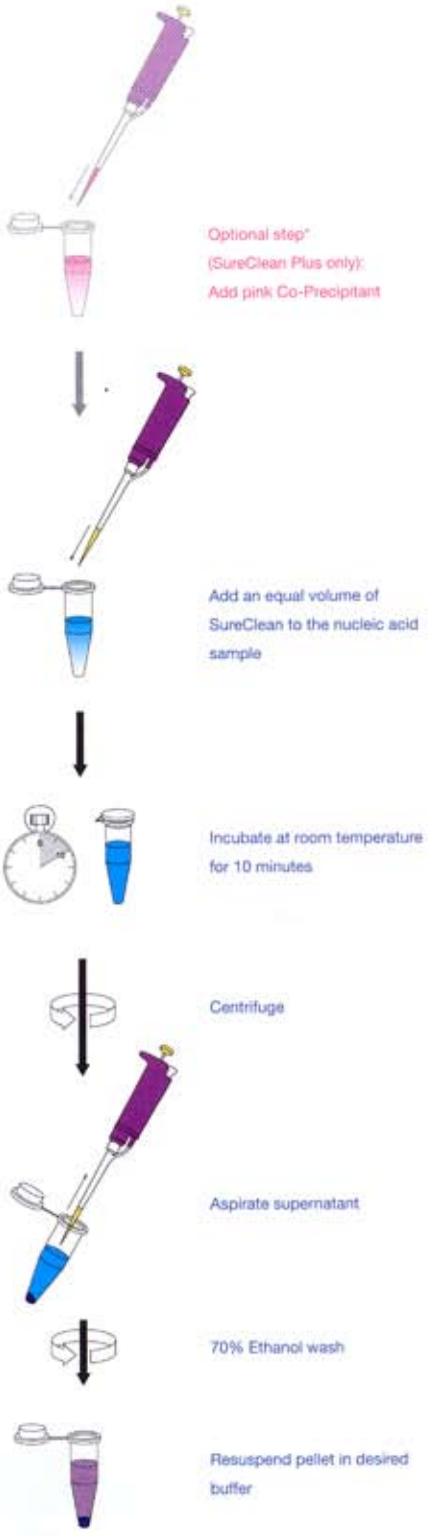
PRODUCT	PACK SIZE	PACK SIZE	CAT NO.
SureClean	DNA Clean-up	1 x 5ml	BIO-37042
SureClean	DNA Clean-up	5 x 5ml	BIO-37046
SureClean Plus	with Pink Co-precipitant	1 x 5ml	BIO-37047
SureClean Plus	with Pink Co-precipitant	5 x 5ml	BIO-37048

Features

- Column-free PCR clean-up
- Recovery rates of up to 98%
- Optional Pink Co-Precipitant allows easy visualisation of DNA pellet
- Suitable for DNA, cDNA, or dsRNA purification
- Products are suitable for immediate downstream applications

Applications

- PCR clean-up
- Removes primers, primer-dimers, dNTPs, restriction enzymes
- Purifying or concentrating DNA
- Purifying dsRNA



Description

SureClean is a novel, inexpensive solution, which provides a column-free method for nucleic-acid purification. Using a simple and rapid procedure, SureClean can be used to purify or concentrate DNA or dsRNA from PCR reactions or any enzymatic digests. This method is easy to follow, combining convenience, speed and excellent recovery rates.

Simple, Flexible and Column-free Protocol

SureClean removes proteins (such as restriction enzymes, polymerases, etc), primers/ primer-dimers and dNTPs. A very straightforward protocol allows the precipitation of nucleic acids ≥100bp without the need for organic solvents, glass milk or expensive spin-columns. Unlike many column-based methods, SureClean maximises recovery with nucleic acid solutions, whether of low, medium or high concentration. SureClean purifies nucleic acid without the use of chaotropic salts (which often contribute to denaturation of DNA duplex). SureClean enables the researcher to re-suspend the cleaned-up nucleic acids in any buffer and volume of choice, thus permitting the purification process to be tailored specifically to suit the experiment.

Optimised Nucleic Acid Recovery

SureClean has been tailored to maximise the amount of nucleic acid recovered after purification, providing up to 98% recovery of the original sample for immediate downstream applications, such as cloning and sequencing. SureClean exhibits great versatility, achieving unsurpassed recovery rates, independently of the amount of nucleic acid or its concentration.

Optional Pink Co-Precipitant

SureClean Plus incorporates a pink co-precipitant that offers the distinct advantage of easy visualisation of the purified pellet, since this acquires a pink colour. Pink co-precipitant (Cat No. BIO-37075) is part of the Bioline range of linear polyacrylamides designed to aid recovery of nucleic acids. Pink co-precipitant is specially treated, does not contain any detectable amounts of nucleic acids, and is suitable for use in standard PCR, RT-PCR, and other enzymatic reactions.

Nucleic acids purified with SureClean Plus are not suitable for spectral determination of nucleic acids within the 260nm - 280nm range. Should you wish to carry out such experiments, the use of SureClean is recommended.

Storage Conditions

SureClean solution can be stored at room temperature for 12 months. Do not freeze.
Pink co-precipitant can be stored at +4°C for up to 12 months. For short-term storage, room temperature is also possible. For long-term storage (up to 2 years), pink co-precipitant should be stored at -20°C. Avoid exposure of pink co-precipitant to light.

Associated Products

PRODUCT	CAT NO.	CHAPTER
ACCUZYME	BIO-21052	Enzymes for Molecular Biology
AccuSure	BIO-21069	Enzymes for Molecular Biology
IMMOLASE	BIO-21048	Enzymes for Molecular Biology
BIO-X-ACT Short	BIO-21065	Enzymes for Molecular Biology
BIO-X-ACT Long	BIO-21050	Enzymes for Molecular Biology
Diamond	BIO-21059	Enzymes for Molecular Biology
BIOTAQ	BIO-21060	Enzymes for Molecular Biology
MangoTaq	BIO-21078	Enzymes for Molecular Biology
Agarose	BIO-41026	Reagents for Molecular Biology
18.2MΩ Water	BIO-37080	Reagents for Molecular Biology

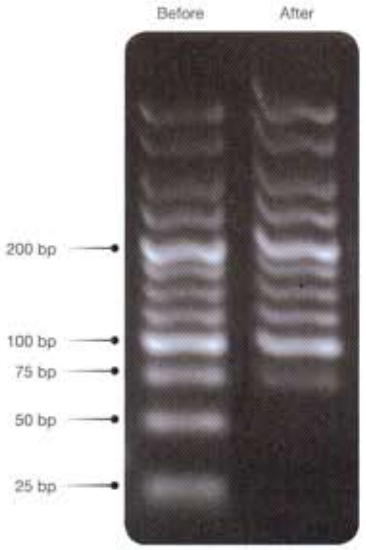


Fig 7.1 DNA from a MW Marker (Hyperladder V) treated with SureClean.
The gel picture demonstrates that the smallest bands (50 and 25 bp) are removed after the purification process.

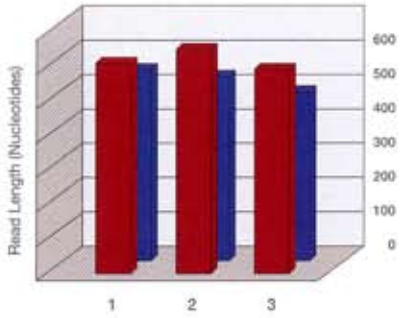


Fig 7.2 Chart showing the length of read from a sequencing reaction using SureClean (red) and competitor Q's spin columns (blue).

Proteinase K

PRODUCT	PACK SIZE	CAT NO.
Proteinase K	100mg	BIO-37037
Proteinase K	1000mg	BIO-37038
Proteinase K Solution	5ml	BIO-37084
Proteinase K Solution	5 x 5ml	BIO-37085

Features

- Broad-spectrum serine protease
- Active under denaturing conditions
- Stable at high temperatures
- Molecular biology grade
- Available as powder and stabilised stock solution

Applications

- Inactivation of RNases/DNases during nucleic acid extraction
- Protein modification
- General protein digestion

Description

Proteinase K is an enzyme used to digest most proteins in molecular-biological techniques. The enzyme may be used at 56°C for up to 4 hours, or 37°C for overnight incubations. Proteinase K is available as a powder or as a stabilised stock solution (20mg/ml). Proteinase K solution is stabilised with a specially formulated buffer, and can be used directly from the freezer.

Storage Conditions

Proteinase K can be stored for 12 months at -20°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Set	BIO-39025	Nucleotides
dNTP Mix	BIO-39028	Nucleotides
2x PolyMate Additive	BIO-37041	Reagents for Molecular Biology
Hyper Ladder I	BIO-33025	Molecular Weight Markers

X-GAL

PACK SIZE	CAT NO.
1g	BIO-37035

Features

- Extremely pure, 99.5% by TLC
- Intense blue precipitate upon hydrolysis

Applications

- Blue/White cloning systems
- Immunoblotting
- Immunocyto chemical assays
- Microbiology and cell culture media

Description

5-bromo-4-chloro-3-indolyl ß-D-galactopyranoside (X-GAL) is a chromogenic substrate for ß-Galactosidase that forms an intense blue precipitate. It can be used in Molecular Biology to detect the gal gene product, and also in Microbiology where it is used to detect micro-organisms which have ß-Galactosidase activity (usually coliforms). It can be combined with the R-substrates to differentiate between two species of organisms on the same plate. X-GAL is soluble in N, N-dimethylformamide.

Storage Conditions

X-GAL can be stored for 12 months at -20°C. Store protected from light.

Associated Products

PRODUCT	CAT NO.	CHAPTER
IPTG	BIO-37036	Cloning
X-GAL/IPTG	BIO-37086	Cloning
Thermo DNA Ligase	BIO-27045	Cloning
T4 DNA Ligase	BIO-27026	Cloning

IPTG

PRESENTATION	PACK SIZE	CAT NO.
IPTG	5g	BIO-37036
IPTG Solution	10ml	BIO-37082
IPTG Solution	5 x 10ml	BIO-37083

Features

- Induces *E.coli lac* operon activity
- >99.6% by HPLC
- Available as powder and stabilised stock solution

Applications

- Blue/White colony screening
- Induction of *lac* operon for protein expression

Description

Isopropyl-ß-D-thiogalactopyranoside (IPTG) is a chemical analogue of lactose. Genes controlled by the *lac* or *tac* promoter/operator sequences are expressed to high levels in the presence of IPTG. IPTG is available as a powder or as a 1M stock solution (240mg/ml). We recommend using IPTG Solution at a concentration of 0.1mM to 1mM.

Storage Conditions

IPTG can be stored for 12 months at -20°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
X-GAL	BIO-37035	Cloning
X-GAL/IPTG	BIO-37086	Cloning
Thermo DNA Ligase	BIO-27045	Cloning
Quick-Stick Ligase	BIO-27027	Cloning

X-GAL/IPTG Solution

PACK SIZE	CAT NO.
10ml	BIO-37086

Features

- Convenient ready-to-use solution
- Ideal for Blue/White colony screening
- Compatible with all commonly used *lacZ* expression systems

Description

X-GAL/IPTG Solution is a ready-to-use solution of 40mg/ml X-GAL (5-bromo-4-chloro-3-indolyl ß-D-galactopyranoside) and 32mg/ml IPTG (Isopropyl-ß-D-1-thiogalactopyranoside), in a non-hazardous and non-toxic solvent (N-methyl-pyrrolidon). We recommend using X-GAL/IPTG Solution at a 1000x concentration. The X-GAL/IPTG Solution saves time and money, since no extra sterilisation is necessary, and it can be used directly from the freezer. The solution provides optimal blue/white colony screening and is compatible with all commonly used *lacZ* expression systems in bacteria, yeast and mammals.

Storage Conditions

X-GAL/IPTG Solution can be stored for 12 months at -20°C. Store protected from light.

Associated Products

PRODUCT	CAT NO.	CHAPTER
X-GAL	BIO-37035	Reagents for Molecular Biology
IPTG	BIO-37036	Reagents for Molecular Biology
Thermo DNA Ligase	BIO-27045	Cloning
Quick-Stick Ligase	BIO-27027	Cloning
T4 DNA Ligase	BIO-27026	Cloning

Agarose (DNase/RNase-free)

PACK SIZE	CAT NO.
100g	BIO-41026
500g	BIO-41025

Features

- Excellent value and clarity
- High gel strength (>1500g/cm²)
- DNase/RNase-free

Applications

- DNA/RNA Electrophoresis
- Ideal for separating nucleic acids of a wide range of sizes, especially large fragments (>10Kb)

Description

Bioline Agarose (DNase/RNase-free) is an extremely pure, high molecular grade agarose powder that has been extensively tested for RNase contamination, and is suited to DNA and RNA electrophoresis. The high gel strength (>1500g/cm²) means that gels as low as 0.5% are practicable.

High Resolution Electrophoresis

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Set	BIO-39025	Nucleotides
2x PolyMate Additive	BIO-37041	Reagents for Molecular Biology
BIOTAQ	BIO-21060	Enzymes for Molecular Biology
RNA Loading Buffer	BIO-38025	Molecular Weight Markers

Agarose Tablets (DNase/RNase-free)

PACK SIZE	CAT NO.
150g	BIO-41028
300g	BIO-41027

Features

- Convenient and time saving
- Greater gel-to-gel consistency
- Safer and cleaner to use
- DNase/RNase-free

Applications

- DNA/RNA Electrophoresis
- Ideal for separating nucleic acids of a wide range of sizes

Description

Bioline no-mess Agarose Tablets (DNase/RNase-free) are designed to be cleaner, safer and more convenient than powdered agarose. Each tablet contains a pre-determined amount of agarose (0.5g agarose per tablet), eliminating the need to weigh out loose agarose powder. Just add the appropriate number of tablets to your buffer, incubate at room temperature for five minutes, heat the solution and then prepare your gel as normal. Bioline Agarose Tablets have the same specification as Bioline Agarose (DNase/RNase-free) powder and are suitable for all routine applications.

Associated Products

PRODUCT	CAT NO.	CHAPTER
dNTP Mix	BIO-39028	Nucleotides
HyperLadder I	BIO-33025	Molecular Weight Markers
Coloured DNA Loading Buffers	BIO-37070	Reagents for Molecular Biology
IMMOLASE	BIO-21048	Enzymes for Molecular Biology

Co-Precipitants

PRODUCT	CONC.	PACK SIZE	CAT NO.
Glycogen	20mg/ml	1ml	BIO-37077
Co-Precipitant Colourless	5mg/ml	1.5ml	BIO-37074
Co-Precipitant Pink	5mg/ml	1.5ml	BIO-37075

Features

- Close to 100% nucleic acid recovery
- Effective for fragments ≥25bp
- Suitable for sequencing
- Free from DNA, RNA and protein
- Increases pellet mass and visibility
- Minimises pellet loss
- Additional buffer supplied

Applications

- DNA and RNA recovery

Description

Bioline Co-Precipitants can be used for the quantitative recovery of small amounts of nucleic acids within dilute solutions. The Co-Precipitants Glycogen (isolated from Oyster) and Linear Polyacrylamides (colourless and pink) aid recovery of nucleic acids during salt/alcohol precipitations. Co-Precipitants are suitable for most applications, including the precipitation of DNA for sequencing, DNA after enzymatic manipulations and RNA from different sources. Bioline Co-Precipitants are free of nucleic acids, therefore all resulting precipitates are suitable for standard PCR, RT-PCR and other enzymatic reactions.

Glycogen precipitant is free of background polynucleotides, DNA and RNA binding proteins, proteases, DNases and RNases. However, some enzymatic reactions may be inhibited by the presence of polysaccharides.

Colourless Polyacrylamide is the only Co-Precipitant suitable for spectral determination of nucleic acid within the 260nm-280nm range.

Storage Conditions

Co-Precipitants can be stored for 18 months at -20°C.

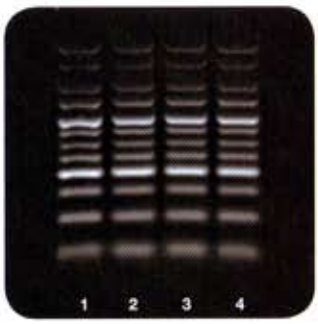


Fig 7.3 20µl of HyperLadder V compared with 20µl of precipitated HyperLadder V resuspended in 20µl of TE

Lane 1: HyperLadder V
Lane 2: With Bioline Glycogen
Lane 3: With Co-Precipitant Colourless
Lane 4: With Co-Precipitant Pink

Associated Products

PRODUCT	CAT NO.	CHAPTER
SureClean Plus	BIO-37047	Reagents for Molecular Biology
Agarose	BIO-41026	Reagents for Molecular Biology
HyperLadder I	BIO-33025	Molecular Weight Markers

Sheared DNA for Hybridisation

PACK SIZE	CONC.	CAT NO.
5ml	10mg/ml	BIO-37078

Features

- DNase/RNase-free
- Ready-to-use solution

Applications

- Southern and Northern hybridisation

Description

Sheared DNA is a molecular biology grade solution designed for direct use as a blocking agent, in Southern and Northern blotting experiments, in order to prevent non-specific DNA hybridisation. Sheared DNA is prepared from fish sperm (Cod), treated with SDS and Proteinase K and extracted with phenol/chloroform. The Sheared DNA fragments are smaller than 1Kb and tested for molecular weight size and absence of DNase/RNase activities.

Concentration

10mg/ml

Storage Conditions

Sheared DNA can be stored for 12 months at -20°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
Agarose	BIO-41026	Reagents for Molecular Biology
Proteinase K	BIO-37037	Reagents for Molecular Biology

Half-Dye Mix

PACK SIZE	CAT NO.
250 Templates	BIO-36025
1000 Templates	BIO-36026

Features

- Reduces autosequencing costs
- Ready-to-use format
- No optimisation required

Applications

- Autosequencing of plasmid and PCR templates

Description

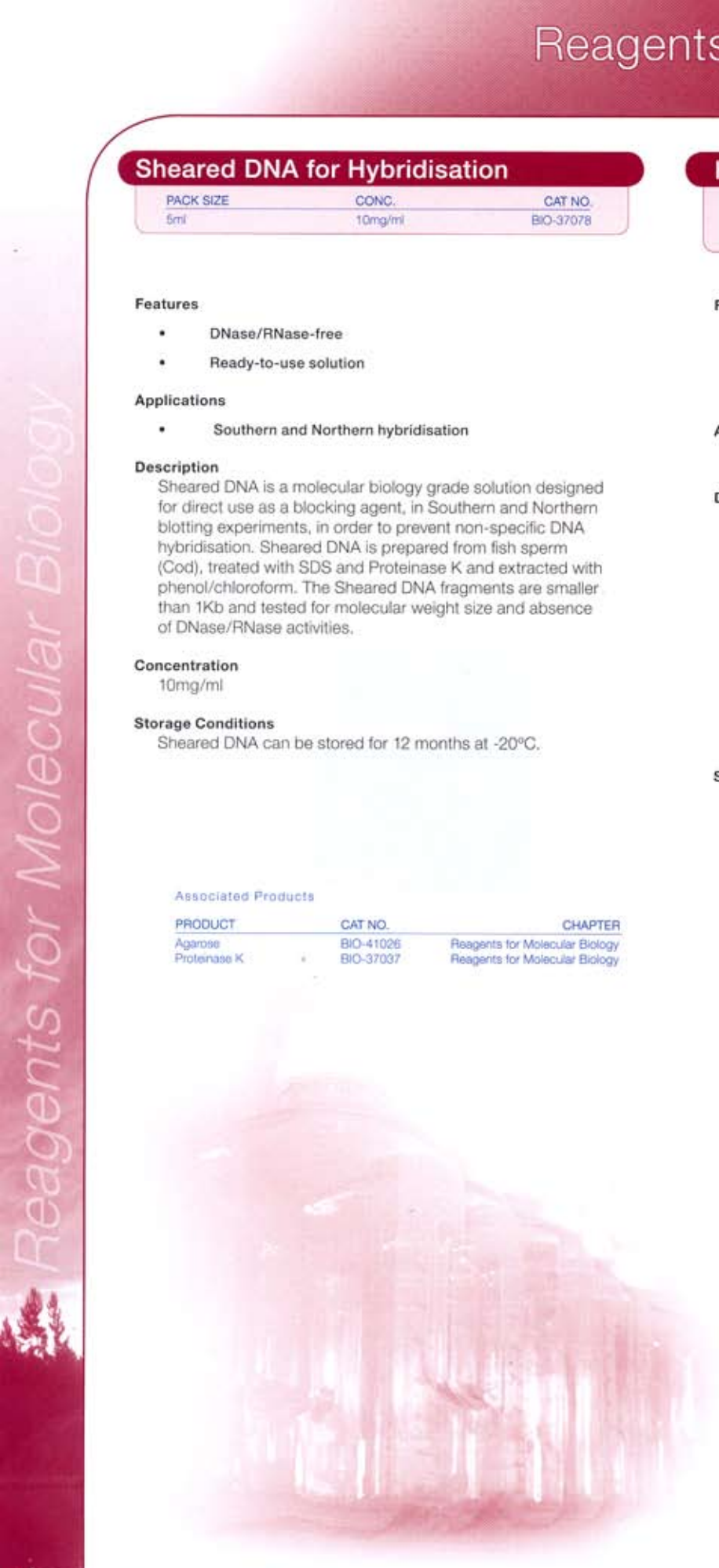
Half-Dye Mix reduces the costs involved in autosequencing, by reducing the amount of ABI™ BigDye Terminator needed in a reaction. Autosequencing reactions can leave up to 80% of dye terminators unused, and these normally require removal prior to sequencing. Half-Dye Mix provides the necessary reaction conditions to allow for a lower proportion of dye terminator in the reaction mix, so reducing the amount of unused dye terminators that require removal, and allowing many more reactions from every purchase of dye terminator. Half-Dye Mix works by providing optimal buffer conditions, which allow up to a 5-fold dilution of dye terminator without any loss of sequencing quality.

Storage Conditions

Half-Dye Mix can be stored for 6 months at -20°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
ACCUZYME	BIO-21051	Enzymes for Molecular Biology
AccuSure	BIO-21068	Enzymes for Molecular Biology
dNTP Set	BIO-39025	Nucleotides



PolyMate Additive

PACK SIZE	CAT NO.
2 x 1.2ml	BIO-37041

Features

- Dramatically improves specificity and yield
- Compatible with all commercially available thermostable DNA polymerases
- Ideal for "difficult" templates
- Reduces smearing and background

Applications

- Enhancing the performance and specificity of any thermostable DNA polymerase in enzyme reactions

Description

PolyMate is a special 2x additive for use in reactions involving any thermostable DNA polymerase, and is designed to dramatically improve reaction specificity. PolyMate provides an optimised composition of reagents, and is ideally suited to dirty/difficult templates with GC or AT-rich DNA, repetitive sequences or difficult melting profiles. PolyMate acts as a melting agent by allowing the DNA polymerase and oligonucleotides greater access to the template DNA. PolyMate does not contain magnesium, dNTPs, or buffer components. In some cases it may be necessary to optimise the Magnesium concentration.

Note

PolyMate should not be used in combination with any other additives for polymerase reactions.

Concentration

2x

Storage Conditions

PolyMate Additive can be stored for 6 months at -20°C.

Fig 7.5 PolyMate assayed with three different polymerases on a 201bp GC-Rich fragment >66% from Human TGF - b gene

Lane 1: HyperLadder II
Lane 2: Treated with PolyMate
Lane 3: Without PolyMate

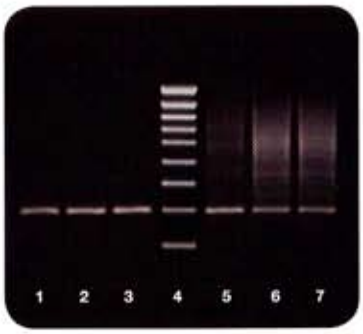


Fig 7.6 PCR of a 201bp GC-Rich fragment >66% from Human TGF - 8 gene

Lane 1-3: With PolyMate & 1.5mM, 2.0mM & 2.5mM $MgCl_2$ respectively
Lane 4: HyperLadder IV
Lane 5-7: Without PolyMate or $MgCl_2$

Fig 7.7 PolyMate assayed with three different polymerases on a 234bp GC-Rich fragment >68% from Human ApoE gene

Lane 1: HyperLadder II
Lane 2: Treated with PolyMate
Lane 3: Without PolyMate

Associated Products

PRODUCT	CAT NO.	CHAPTER
ACCUZYME	BIO-21051	Enzymes for Molecular Biology
AccuSure	BIO-21068	Enzymes for Molecular Biology
IMMOLASE	BIO-21046	Enzymes for Molecular Biology
Diamond	BIO-21058	Enzymes for Molecular Biology
BIOTAQ	BIO-21040	Enzymes for Molecular Biology

Hi-Spec Additive

PACK SIZE	CAT NO.
3 x 1.2ml	BIO-37032

Features

- Eliminates background smears and spurious bands
- Improves specificity
- Compatible with all commercially available DNA polymerases
- Ideal for "difficult" templates

Applications

- Improving the specificity of any DNA polymerase in enzyme reactions

Description

Hi-Spec Additive is a popular compound designed to eliminate unwanted by-products, such as background smears and spurious bands, during DNA amplification. Hi-Spec Additive is ideally suited to difficult templates with GC-rich regions or repetitive sequences.

Concentration

5x

Storage Conditions

Hi-Spec Additive can be stored for 6 months at -20°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
BIO-X-ACT Long	BIO-21049	Enzymes for Molecular Biology
BIO-X-ACT Short	BIO-21064	Enzymes for Molecular Biology

OptiBuffer

PACK SIZE	CAT NO.
1 x 1.2ml	BIO-37030
3 x 1.2ml	BIO-37031

Features

- Improves enzyme thermostability
- Increases enzyme half-life (*in vitro*)
- Replaces standard buffers
- High DNA yield with low background
- High processivity and fidelity

Applications

- Improving the performance of any thermostable DNA polymerase in assays

Description

OptiBuffer is a non-Tris incubation buffer for any polymerase applications where optimal conditions are essential to achieve a high yield, without giving rise to non-specific background. OptiBuffer provides an optimal pH (7.8-8.0 at 70°C-72°C) throughout the whole reaction, owing to a buffering capacity that is much higher than that of conventional Tris-HCl-based buffers. This is essential for the processivity and fidelity of thermostable DNA polymerases. OptiBuffer will enhance results with all templates, but is particularly useful for difficult templates and for the synthesis of long regions. OptiBuffer is simply substituted in place of the incubation buffer supplied with thermostable polymerases, and provides the easiest way to improve the performance of DNA polymerase assays.

Concentration

10x

Storage Conditions

OptiBuffer can be stored for 6 months at -20°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
HyperLadder I	BIO-33025	Molecular Weight Markers
BIO-X-ACT Long	BIO-21049	Enzymes for Biology

10x ROX Reference Buffer

PACK SIZE	CAT NO.
500µl	BIO-37087

Fluorescence Normalization

Description

10x ROX Reference Buffer is a fluorescent dye which is suitable for signal normalization.

Storage conditions

10x ROX Reference Buffer can be stored for 6 months at -20°C. Store protected from light.

RNA Loading Buffers

PRODUCT	CONC.	PACK SIZE	CAT NO.
RNA Loading Buffer with Ethidium Bromide	2x	1ml	BIO-38025
RNA Loading Buffer without Ethidium Bromide	2x	1ml	BIO-38026

Features

- **Available with or without Ethidium Bromide**
- **Ready-to-use solution**

Applications

- **Northern Blot analysis**
- **Agarose gel electrophoresis**

Description

The new Bioline 2x RNA Loading Buffers maintain the denatured state of the RNA during electrophoresis. The ready-to-use buffer solutions are available either with or without ethidium bromide. RNA Loading Buffer with ethidium bromide includes ethidium bromide in the buffer, therefore it is not necessary to add it to the gel.

If the RNA is to be used in a Northern Blot we recommend using RNA Loading Buffer without ethidium bromide. Ethidium bromide reduces hybridisation efficiency once the RNA is transferred to a membrane.

We recommend using RNA Loading Buffer on MOPS agarose gels. The buffer can also be used on formaldehyde, glyoxal, agarose gels and acrylamide urea gels.

Concentration

2x

Storage Conditions

RNA Loading Buffers can be stored for 12 months at -20°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
RiboLadder Short	BIO-33060	Molecular Weight Markers
RiboLadder Long	BIO-33061	Molecular Weight Markers
Agarose	BIO-41025	Reagents for Molecular Biology

10x NH_4 Buffer

PACK SIZE	PACK SIZE	CAT NO.
160mM $(NH_4)_2SO_4$, 670mM Tris-HCl (pH8.8 at 25°C), 0.1% Tween-20	3 x 1.2ml	BIO-37025

10x NH_4 + $MgCl_2$ Buffer

PACK SIZE	PACK SIZE	CAT NO.
160mM $(NH_4)_2SO_4$, 670mM Tris-HCl (pH 8.8 at 25°C), 0.1% Tween-20, 15mM $MgCl_2$	3 x 1.2ml	BIO-37064

10x KCl Reaction Buffer

PACK SIZE	PACK SIZE	CAT NO.
500mM KCl, 100mM Tris-Cl (pH 8.8 at 25°C), 15mM $MgCl_2$, 1% Triton X-100 15mM	3 x 1.2ml	BIO-37066

50mM $MgCl_2$ Solution

PACK SIZE	PACK SIZE	CAT NO.
$MgCl_2$ 50mM in water, DNase and RNase-free	3 x 1.2ml	BIO-37026

Ultra-Pure Water

High quality, molecular biology grade water is an essential reagent for all areas of molecular biology, and is especially critical for RNA manipulation and analysis. Poor quality water can often contain contaminants such as DNA, RNA, nucleases and microorganisms. RNase contamination will jeopardise any work involving RNA, and is extremely difficult to eliminate from biological samples.

Bioline ultra-pure 18.2MΩ Water and the new DEPC-treated Water are extremely pure molecular biology grade water, and are suitable for all PCR, RT-PCR, real-time PCR and electrophoresis applications. For all applications involving RNA we recommend using DEPC-treated Water, which is chemically treated to destroy any RNase contamination.

DEPC-treated Water

PACK SIZE	CAT NO.
10 X 10ml	BIO-38030
1 Litre	BIO-38031

DEPC-treated Water is ideal for use in all RNA work. DEPC-treated Water is prepared by treating ultra-pure 18.2MΩ Water with diethylpyrocarbonate (DEPC), and is then autoclaved to inactivate the DEPC.

Storage Conditions

DEPC-treated Water can be stored for 12 months at -20°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
RiboLadder Short	BIO-33060	Molecular Weight Markers
10x MOPS Electrophoresis Buffer	BIO-38027	RNA Analysis
RNA Loading Buffer	BIO-38025	Molecular Weight Markers
BioScript	BIO-27036	RNA Analysis

Water, 18.2MΩ (DNase/RNase-free)

PACK SIZE	CAT NO.
10 X 10ml	BIO-37080

Associated Products

PRODUCT	CAT NO.	CHAPTER
T7 RNA Transription	BIO-21072	RNA Analysis
Thermo DNA Ligase	BIO-27045	Cloning
Quick-Stick Ligase	BIO-27028	Cloning
Agarose Tablets	BIO-41027	Reagents for Molecular Biology

Coloured DNA Loading Buffers

COMPOSITION	PACK SIZE	CAT NO.
DNA Loading Buffer, Blue	2 x 1ml	BIO-37045
DNA Loading Buffer, Red	2 x 1ml	BIO-37068
Tri-Colour DNA Loading Buffer	2 x 1ml	BIO-37070

Applications

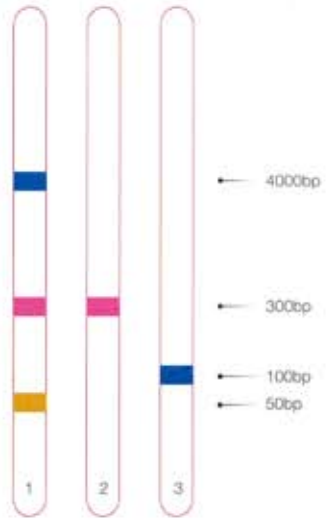
- **To monitor migration rate during agarose electrophoresis**

Description

5x Coloured DNA Loading Buffers

Associated Products

PRODUCT	CAT NO.	CHAPTER
Agarose Tablets	BIO-41028	Reagents for Molecular Biology
HyperLadder I	BIO-33025	Molecular Weight Markers
BIOTAQ Red	BIO-21041	Enzymes for Molecular Biology
dNTP Set	BIO-39025	Nucleotides



Graphic representation of Coloured DNA Loading Buffers in 2% agarose gel

Lane1: Tri-Colour DNA Loading Buffer
Lane 2: DNA Loading Buffer, Red
Lane 3: DNA Loading Buffer, Blue

Mouse Genomic DNA

PACK SIZE	CAT NO.
500µl	BIO-35027

Features

- Highly purified
- Fragments are >50Kb

Applications

- Southern blotting
- Genomic library construction
- Control template

Description

Mouse Genomic DNA is highly purified and isolated from *Total Mouse Genomic* DNA. The average length of the DNA is greater than 50Kb and is suitable for Southern blotting, genomic library construction and as a control template.

Concentration

200ng/µl

Storage Conditions

Mouse Genomic DNA should be stored at +4°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
Agarose	BIO-41026	Reagents for Molecular Biology
HyperLadder I	BIO-33025	Molecular Weight Markers

Human Genomic DNA

PACK SIZE	CAT NO.
500µl	BIO-35025

Features

- Highly purified
- Fragments are >50Kb

Applications

- Southern blotting
- Genomic library construction
- Control template

Description

Human Genomic DNA is highly purified and isolated from human placenta. The average length of the DNA is greater than 50Kb and is suitable for Southern blotting, genomic library construction and as a control template.

Concentration

200ng/µl

Storage Conditions

Human Genomic DNA should be stored at +4°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
Agarose Tablets	BIO-41028	Reagents for Molecular Biology
18.2MΩ Water	BIO-37080	Reagents for Molecular Biology

Rat Genomic DNA

PACK SIZE	CAT NO.
500µl	BIO-35026

Features

- Highly purified
- Fragments are >50Kb

Applications

- Southern blotting
- Genomic library construction
- Control template

Description

Rat Genomic DNA is highly purified and isolated from *Rattus norvegicus* brain tissue. The average length of the DNA is greater than 50Kb and is suitable for Southern blotting, genomic library construction and as a control template.

Concentration

200ng/µl

Storage Conditions

Rat Genomic DNA should be stored at +4°C.

Associated Products

PRODUCT	CAT NO.	CHAPTER
Agarose	BIO-41026	Reagents for Molecular Biology
Agarose Tablets	BIO-41028	Reagents for Molecular Biology

The Genetic Code

A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
5' GCA	CGA	AAC	GAC	UGC	CAA	GAA	GGA	CAC	AUA	CUA	AAA	AUG	UUC	CCA	UCA	ACA	UGG	UAC	GUA 3'
C	C	U	U	U	G	G	C	U	C	C	G		U	C	C	C		U	C
G	G						G		U	G				G	G	G			G
U	U						U			U				U	U	U			U
	or									or					or				
	AGA									UUA					AGC				
	G									G					U				

The Genetic Code

1st Position	2nd Position U		2nd Position C		2nd Position A		2nd Position G		3rd Position
	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys	C
	UUA	Leu	UCA	Ser	**UAA**	Stop	**UGA**	Stop	A
	UUG	Leu	UCG	Ser	**UAG**	Stop	UGG	Trp	G
	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC	Leu	CCC	Pro	CAC	His	CGC	Arg	C
	CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg	A
	CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg	G
	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser	C
	AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg	A
	AUG	***Met***	ACG	Thr	AAG	Lys	AGG	Arg	G
	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
	GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly	C
	GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly	A
	GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly	G

The codons are read in the 5' - 3' direction.
Termination codons are in bold.
AUG start codon is bold italic.

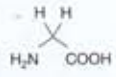
Amino Acids: Three letter abbreviations & one-letter symbols

Amino Acid	Three-Letter Abbreviation	One-Letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Asparagine or aspartic acid	Asx	B
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic Acid	Glu	E
Glutamine or glutamic acid	Glx	Z
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

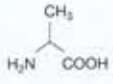
The average molecular weight of an amino acid is 110Da.
Dalton (Da) is an alternate name for the atomic mass unit and Kilodalton (KDa) is 1,000 daltons.
Thus a protein with a mass of 64KDa has a molecular weight of 64,000 grams per mole.

Amino Acid Structures

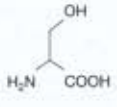
Amino Acid Structures are accompanied by three- and one-letter codes, residue molecular weight (actual molecular weight minus water) and side-chain pKa where appropriate.



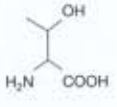
Glycine (GLY, G)
MW: 57.05



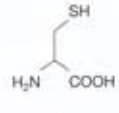
Alanine (Ala, A)
MW: 71.09



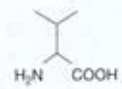
Serine (Ser, S)
MW: 87.08, pK_a ~ 16



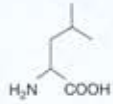
Threonine (Thr, T)
MW: 101.11, pK_a ~ 16



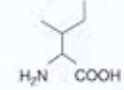
Cysteine (Cys, C)
MW: 103.15, pK_a = 8.35



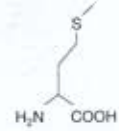
Valine (Val, V)
MW: 99.14



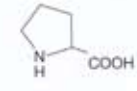
Leucine (Leu, L)
MW: 113.16



Isoleucine (Ile, I)
MW: 113.16

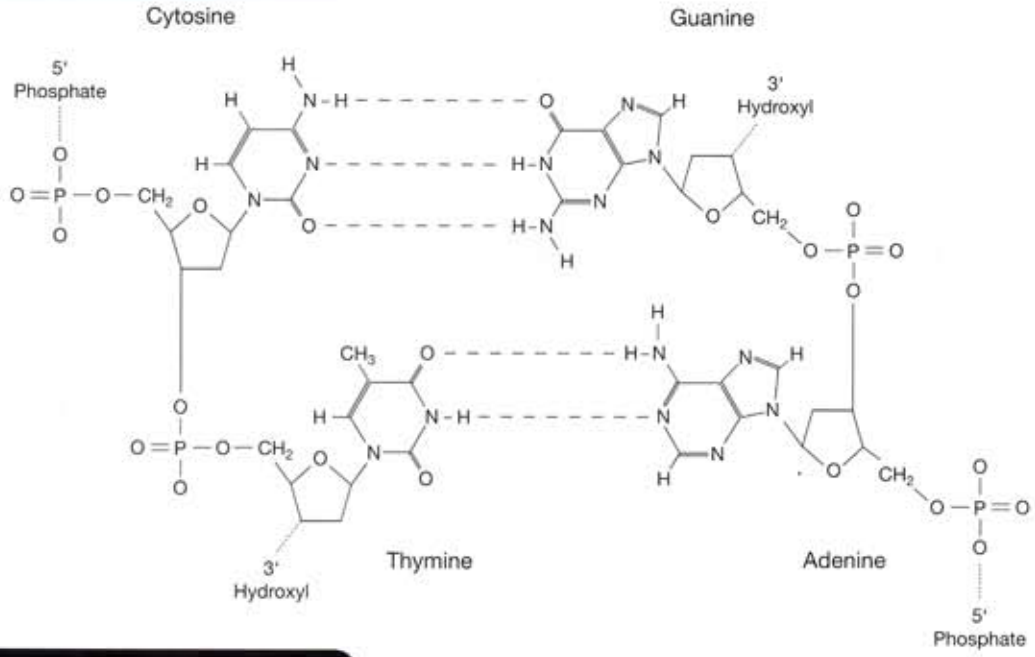


Methionine (Met, M)
MW: 131.19



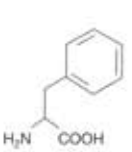
Proline (Pro, P)
MW: 97.12

Nuclear Base Pairing Structural Diagram

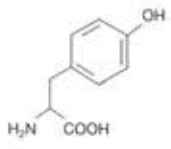


IUPAC Nucleotide Ambiguity Codes

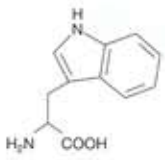
Y = T or C (pyrimidine)
R = G or A (purine)
M = A or C (amino)
K = G or T (keto)
S = G or C (strong interaction: 3 H bonds)
W = A or T (weak interaction: 2 H bonds)
B = G or T or C (not-A)
V = G or C or A (not-T, not-U)
D = G or A or T (not-C)
H = A or C or T (not-G)
N = G or A or T or C (unknown nucleotide)



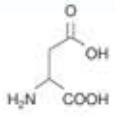
Phenylalanine (Phe, F)
MW: 147.18



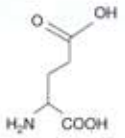
Tyrosine (Tyr, Y)
MW: 163.18



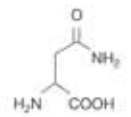
Tryptophan (Trp, W)
MW: 186.21



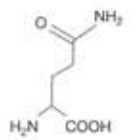
Aspartic Acid (Asp, D)
MW: 115.09, pK_a = 3.9



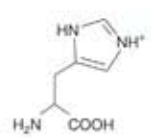
Glutamic Acid (Glu, E)
MW: 129.12, pK_a = 4.07



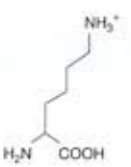
Asparagine (Asn, N)
MW: 114.11



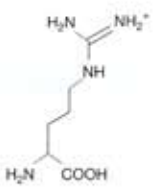
Glutamine (Gln, Q)
MW: 128.14



Histidine (His, H)
MW: 137.14, pK_a = 6.04



Lysine (Lys, K)
MW: 128.17, pK_a = 10.79



Arginine (Arg, R)
MW: 156.19, pK_a = 12.48

Lengths/Molecular Weights of Common Nucleic Acids

Nucleic Acid	Number of Nucleotides	Molecular Weight *
lambda DNA	48502 (dsDNA)	3.2×10^7
pBR322 DNA	4361 (dsDNA)	2.8×10^6
28S rRNA	4800	1.6×10^6
23S rRNA (*E.coli*)	2900	1.0×10^6
18S rRNA	1900	6.5×10^5
16S rRNA (*E.coli*)	1500	5.1×10^5
5S rRNA (*E.coli*)	120	4.1×10^4
tRNA (*E.coli*)	75	2.5×10^4

*Molecular weights based on actual sequence

Standards
1) Average MW of a dsDNA base pair = 660
2) Average MW of a ssDNA base = 330
3) Average MW of an RNA base = 340

References
Daniels, D.L *et al.* (1983) *Appendix II: Complete annotated lambda sequence.* In: *Lambda II,* ed., R.W. Hendrix et al., Cold Spring HarborLaboratory, Cold Spring Harbor, NY, 519

Sutcliffe, J.G. (1978) *Proc. Natl. Acad. Sci.* USA 75, 3737.
Sutcliffe, J.G. (1979) *Cold Spring Harbour Symp. Quant. Biol.* 43, 77.

Ribosomal RNA sizes from various species

SPECIES	16S rRNA	18S rRNA	23S rRNA	25S rRNA	26S rRNA	28S rRNA
Human	-	1.9	-	-	-	5.0
Mouse	-	1.9	-	-	-	4.7
Drosophila	1.5	2.0	-	-	-	4.1*
Tobacco Leaf	-	1.9	2.9	3.7	-	-
Yeast	-	2.0	-	-	3.8	-
E. Coli	1.5	-	2.9	-	-	-
Xenopus	1.8	-	-	-	-	4.0

*Drosophila 28S rRNA is processed into 2 fragmants that migrate in a similar fashion to the 18S rRNA

An RNase-free world

The most critical factor for any work involving RNA is a clean environment. RNA is subject to digestion by a class of enzymes called ribonucleases that can be found everywhere, they are very hardy and difficult to inactivate. When planning to do any work with RNA we would recommend considering the following points:

- All equipment used should either be sterile disposable plasticware which is DNase and RNase-free or pretreated before use, either using one of the many commercially available RNase removal products or soaking in 3% H_2O_2 and rinsing with Ethanol before air drying. This step is often overlooked and it is often assumed that simply autoclaving tips and tubes is sufficient to remove RNase.
- Although many sources of deionised water are RNase-free, we generally recommend using DEPC-treated water for all applications involving RNA. If normal water is to be used, it should be tested by incubating with an RNA sample and run on a gel to check for signs of degradation.
- Gloves are essential for any RNA work as the skin is a major source of RNase contamination.
- Sterile technique is a must when handling any reagents for RNA work; some labs may find it useful to set up a special RNA area with separate pipettes and equipment only used for RNA work.
- A common source of contamination will come directly from your sample. The use of an RNase inhibitor in your reaction can help to overcome this problem. This protein binds to RNases, inhibiting their activity and therefore protecting your valuable RNA.

Physical Properties of ß-Emitting Radionuclides

Radionuclide	Half-Life	Specific Activity: Common Values for Compounds (mCi/mmol)	Daughter Nuclide (stable)
tritium [^{3}H]	12.43 years	10^2 - 10^3	helium-3
carbon-14 [^{14}C]	5,730 years	1 - 10^2	nitrogen-14
sulphur-35 [^{35}S]	87.4 days	1 - 10^6	chlorine-35
phosphorus-33 [^{33}P]	25.5 days	10 - 10^4	sulphur-33
phosphorus-32 [^{32}P]	14.3 days	10 - 10^6	sulphur-32

Physical Properties of γ-Ray and X-Ray Emitting Radionuclides

Radionuclide	Half-Life	Specific Activity: Common Values for Compounds (mCi/mmol)	Daughter Nuclide (stable)
iodine-131 [^{131}I]	8.06 days	10^2 - 10^5	xenon-131
iodine-125 [^{125}I]	60 days	10^2 - 10^4	tellurium-125

Metric Prefixes

Prefix	Symbol	Factor
kilo	K	10^{3}
centi	c	10^{-2}
milli	m	10^{-3}
micro	μ	10^{-6}
nano	n	10^{-9}
pico	p	10^{-12}
femto	f	10^{-15}
atto	a	10^{-18}
zepto	z	10^{-21}

Spectrophotometric Conversions

1 A_{260} unit of double-stranded DNA = 50μg/ml
1 A_{260} unit of single-stranded DNA = 33μg/ml
1 A_{260} unit of single-stranded RNA = 40μg/ml

DNA Molar Conversions

1μg of 1000bp DNA = 1.52pmol (3.03pmol of ends)
1μg of pBR322 DNA = 0.36pmol DNA
1pmol of 1000bp DNA = 0.66μg
1pmol of pBR322 DNA = 2.8μg

Protein/DNA Conversions

1Kb of DNA = 333 amino acids of coding capacity = 37KDa protein
270bp DNA = 10KDa of protein
810bp DNA = 30KDa protein
1.35Kb DNA = 50KDa protein
2.7Kb DNA = 100KDa protein
Average MW of an amino acid = 110 Da

1 Dalton (Da) = 1 gram per mole

Dye Migration: Polyacrylamide Denaturing Gels

Gel %	Bromophenol Blue	Xylene Cyanol
5	35bp	140bp
6	26bp	106bp
8	19bp	75bp
10	12bp	55bp
20	8bp	28bp

Dyes will migrate to the same point as double-stranded DNA of the indicated size in a denaturing polyacrylamide gel.

Adapted from Sambrook, J.,Fritsch, E.F. and Maniatis, T. (1989) In: *Molecular Cloning: A laboratory Manual*,Cold Spring Harbour Laboratory, Cold Spring Harbor, NY.

Dye Migration: Polyacrylamide Nondenaturing Gels

Gel %	Bromophenol Blue	Xylene Cyanol
3.5	100bp	460bp
5	65bp	260bp
8	45bp	160bp
12	20bp	70bp
15	15bp	60bp
20	12bp	45bp

Dyes will migrate to the same point as double-stranded DNA of the indicated size in a nondenaturing polyacrylamide gel.

Adapted from Sambrook, J.,Fritsch, E.F. and Maniatis, T. (1989) In: *Molecular Cloning: A laboratory Manual*,Cold Spring Harbour Laboratory, Cold Spring Harbor, NY.

Dye Migration in 0.5-1.4% Agarose (sizes are approximate)

Xylene cyanol FF	4000bp
Cresol Red	300bp
Bromophenol Blue	100bp
Orange G	50bp

Adapted from Sambrook, J.,Fritsch, E.F. and Maniatis, T. (1989) In: *Molecular Cloning: A laboratory Manual*,Cold Spring Harbour Laboratory, Cold Spring Harbor, NY.

Gel Percentages: Resolution of Linear DNA on Agarose Gels

Recommended % Agarose	Optimum Resolution for Linear DNA (Size of fragments in nucleotides; bp)
0.5	1000-30000
0.7	800-12000
1	500-10000
1.2	400-7000
1.5	200-3000
2	50-2000

pH vs Temperature for Tris Buffer

pH of Tris Buffer (0.05M)		
5°C	25°C	37°C
7.76	7.2	6.91
7.89	7.3	7.02
7.97	7.4	7.12
8.07	7.5	7.22
8.18	7.6	7.3
8.26	7.7	7.4
8.37	7.8	7.52
8.48	7.9	7.62
8.58	8	7.71
8.68	8.1	7.8
8.78	8.2	7.91
8.88	8.3	8.01
8.98	8.4	8.1
9.09	8.5	8.22
9.18	8.6	8.31
9.28	8.7	8.42

Genetic Markers in *E.coli*

Symbol	Description	Effect of Mutation
ara-14	Mutation in arabinose metabolism	Blocks aribinose catabolism
*ara*D	L-ribulose phosphate 4-epimerase mutation; part of an inducible operon araBAD repressed by L-arabinose	Blocks aribinose catabolism
*arg*A	N-Acetylglutamate synthase mutation; inhibited by the presence of arginine	Arginine required from growth in minimal media
*cyc*A	Involved in D-alanine, glycine, D-serine and D-cycloserine transport, and an L-alanine carrier	Mutants cannot use D-alanine as a carbon source
dam	DNA adenine methylase mutation	Blocks methylation of adenine residues in the sequence shown 5'...GmATC...3'
*dap*D	Succinyl-diaminopimelate aminotransferase mutation	Mutant reflects impaired sunthesis of succinyl CoA and needs to be supplemented with succinate or lysine + methionine
dcm	DNA cytosine methylase mutation	Blocks methylation of cytosine in the sequence shown 5'...CmCAGG...3' or 5'...CmCTGG...3'
*deo*C	Deoxyribose-phosphate aldolase mutation	
*deo*R	Regulatory gene mutation allowing constitutive expression of genes for deoxyribose synthesis	Allows efficient propagation of large plasmids
*dut*1	Mutation of deoxyuridine triphosphatase, which catalyses the conversion of dUTP to dUMP and PPi	Mutants are impaired in conversion of dUTP to dUMP, leading to higher dUTP pools that can lead to misincorporation of uracil instead of thymidine. Stable incorporation of dUTP needs mutation in ung gene
*end*A1	DNA-specific endonuclease I mutation	Improves quality of plasmid DNA isolations
*gal*E	Part of the galETK operon that encodes UDP galactose-4-epimerase	Mutant is more resistant to bacteriophage P1 infection
*gal*K	Galactokinase mutation	Blocks catabolism of galactose
*gal*T	Galactose-1-phosphate uridylyltransferase mutation	Blocks catabolism of galactose
*gyr*A96	DNA gyrase mutation	Confers resistance to nalidixic acid
*hfl*A150	Protease mutation which leads to stabilisation of cII gene products	Leads to high frequency of lysogeny by λ phages (1)
*hfl*B	Gene encodes a possible protease component	Mutations lead to high frequency of bacteriophage lambda lysogenisation
hsdR ($r_K^- m_K^+$)	Host DNA restriction and methylation system mutation. Restriction minus, modification positive for the *E.coli* K strain methylation system.	Allows cloning without cleavage of transformed DNA by endogenous restriction endonucleases. DNA prepared from this strain can be used to transform r_K^+ *E.coli* strains.
*hsdS*20 ($r_B^- m_B^-$)	Mutation of specificity determinant for host DNA restriction and methylation system. Restriction minus, modification minus for the *E.coli* B strain methylation system.	Allows cloning without cleavage of transformed DNA by endogenous restriction endonucleases. DNA prepared from this strain is unmethylated by the *hsdS*20 methylases.
lacI^q	Overproduction of the *lac* repressor protein	Leads to high levels of the *lac* repressor protein, inhibiting transcription from the *lac* promoter.
lacY	Galactoside permease mutation	Blocks lactose utilisation
*lacZ*ΔM15	Partial deletion of β-D-galactosidase gene	Allows complementation of β-galactosidase activity by α-complementation sequence in pGEM®-Z Vectors. Allows blue/white selection for recombinant colonies when plated on X-GAL.
*leu*B	β-isopropylmalate dehydrogenase mutation	Requires leucine for growth on minimal media.
Δ(*lon*)	Deletion of *lon* protease	Reduces proteolysis of expressed proteins
*Lys*S	pLysS plasmid is integrated into the host genome	Strains carrying this plasmid will be tet resistant and produce T7 lysozyme, a natural inhibitor of T7 RNA polymerase, thus lowering background transcription of sequences under the control of the T7 RNA polymerase promoter (2)
*mcr*A	Mutation in methylcytosine restriction system	Blocks restriction of DNA methylated at the sequence 5'...GmCGC...3'
*mcr*B	Mutation in methylcytosine restriction system	Blocks restriction of DNA methylated at the sequence 5'...AGmCT...3'
*met*B	Cystathionine γ-synthase mutation	Requires methionine for growth on minimal media
*met*C	Cystathionine beta-lyase mutation; involved in methionine biosynthesis	Methionine required from growth in minimal media
mtl	Mutation in mannitol metabolism	Blocks catabolism of mannitol
*mut*S	Methyl-directed mismatch repair mutation	Prevents repair of the newly synthesised, unmethylated strand
ompT	Mutation of protease VII, an outer membrane protein	Reduces proteolysis of expressed proteins
P2	P2 bacteriophage lysogen present in host	λ phages containing the *red* and *gam* genes of λ are growth inhibited by P2 lysogens (3)
*pro*A	γ-glutamyl phoshate reductase mutation	proA/argD mutant will not block proline synthesis, but will be repressed by arginine. Mutants excrete proline on minimal media and are resistant to proline on minimal media and are resistant to proline analogs. proA/argD/argR triple mutant grows slowly on minimal media +arginine

Genetic Markers in *E.coli* (continued)

Symbol	Description	Effect of Mutation
proAB	Mutations in proline metabolism	Requires proline for growth in minimal media
*rec*A1, *rec*A13	Mutation in recombination	Minimises recombination of introduced DNA with host DNA, increasing stability of inserts. Inserts are more stable in *rec*A1 than *rec*A13 hosts
recB, *recC*, *recD*	Exonuclease V mutations. The Rec BCD trimer (exonuclease V) progressively degrades ssDNA and dsDNA in an ATP-dependent manner to form oligonucleotides; implicated in homologous recombination	Reduces general recombination and affects repair of radiation damage. Allows easier propogation of sequences with inverted repeats
recF	Recombination and repair mutation	Mutant cannot repair daughter strand gaps (post-replicational repair)
relA	ppGpp synthetase I mutation, a novel nucleotide guanosine 5'-diphosphate-3'-diphosphate produced in response to starvation by *relA* ribosomal protein sensing uncharged tRNA	Allows RNA synthesis in the absence of protein synthesis
rha	Utilisation of L-rhamnose, a methylpentose	Blocks rhamnose catabolism
rpsL	Mutation in subunit S12 of 30S ribosome	Confers resistance to streptomycin
sbcB	Exonuclease I mutation	Allows general recombination in *rec*BC mutant strains
strA	Mutant alters ribosome protein S12	Confers resistance to streptomycin
supB, *supC*, *supG*, *supL*, *supM*, *supN*, *supO*	Suppressor mutations	Suppresses ochre (UAA) and amber (UAG) mutations
supD, *supE*, *supF*	Suppressor mutations	Suppresses amber (UAG) mutations
thi-1	Mutation in thiamine metabolism	Thiamine required for growth in minimal media
thr	Threonine biosynthesis mutation	Mutants are obligate threonine auxotrophs
thyA	Thymidylate synthase; dTTP biosynthesis	Mutants are obligate thymidine auxotrophs
Tn5	Transposon	Encodes resistance to kanamycin
Tn10	Transposon	Encodes resistance to tetracycline
tonA	Mutation in outer membrane protein	Confers resistance to bacteriophage T1
traD36	Transfer factor mutation	Prevents transfer of F' episome
trpC	Phosphoribosyl anthranilate isomerase mutation; part of tryptophan biosynthesis pathway	
trpR	*trp*R aporepressor; regulates the biosynthesis of tryptophan and its transport	
tsx	T6 and colicin K phage receptor; outer membrane protein involved in specific diffusion of nucleosides; transports the antibiotic albicidin	Resistant to bacteriophage T6 and colicin K
*ung*1	Uracil-DNA N-glycosylase	Allows uracil to exist in plasmid DNA
xyl-5	Mutation in xylose metabolism	Blocks catabolism of xylose

References
1. Hoyt, A. *et. al.* (1982) *Cell* 31, 565
2. Studier, F.W. (1991) *J. Mol. Biol.* 219, 37-44
3. Kaiser, K. and Murray, N. (1985) In: *DNA Cloning*, Vol. 1, Glover, D., ed., IRL Press Ltd., Oxford, UK.
4. Neidnardt, F. ed. (1996) *Escherichia coli and Salmonella Cellular and Molecular Biology* 2nd ed, ASM Press, Washington , D.C.

Genotypes of Bacterial Strains

Strain	Genotype
BL21	F- *ompT* $hsdS_B(r_B^-m_B^-)$ *gal dcm*
BL21 (DE3)	F- *ompT* $hsdS_B(r_B^-m_B^-)$ *gal dcm* (DE3)
BL21 (DE3) pLysS	F- *ompT* $hsdS_B(r_B^-m_B^-)$ *gal dcm* (DE3) pLysS (Cam^R)
BL21 (DE3) pLysE	F- *ompT* $hsdS_B(r_B^-m_B^-)$ *gal dcm* (DE3) pLysE (Cam^R)
α-Select Competent Cells	*deoR endA1 recA1 relA1 gyrA96 hsdR17*($r_k^-m_k^+$) *supE44 thi-1* Δ(*lacZYA-argF*V169) φ80δ*lacZ*ΔM15 F- γ
ElectroSHOX	F- *mcrA* Δ(*mrr-hsd*RMS-*mcr*BC) φ80*lacZ* ΔM15 Δ*lacX74 recA1 endA1 ara* Δ139 Δ(*ara, leu*)7697 *galU galK* λ- *rpsL* (Str^R) *nupG*
BIO*Blue*	*recA1 endA1 gyrA96 thi-1 hsd*R17($r_k^-m_k^+$) *supE44 relA1 lac* [F' *proAB lacI*^{q}Z ΔM15 Tn10(Tet^r)]
CH3-Blue	F- *mcrA* Δ(*mrr-hsd*RMS-*mcr*BC) φ80*lacZ* ΔM15 Δ*lacX74 recA1 endA1 ara* Δ139 Δ(*ara, leu*)7697 *galU galK* λ- *rpsL* (Str^R) *nupG*