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Protein Chip Kit

User's Guide

Polymer substrate and colorimetric reagents for developing protein microarray.

For research use only.

GENERAL INFORMATION

Product Characteristics

Protein Chip is a ready to use polymer substrate and colorimetric reagents system. The kit provides a complete set of common reagents for making microarray for protein / protein binding based on biotin-streptavidin system and alkaline phosphatase color signal reaction. The kit is designed to combine covalent binding technology and let you to design microarray easily. It is an useful tool using biochip for biological research field as Human medical, Veterinarian, Plant pathology, Breeding etc.
Before using the kit for chip design, please read the following instructions:

Printing

The print buffer (PBS pH 7.2) is designed specifically for use with Protein Chip. This buffer allows maximum binding of the amine to the surface. Additives such as DMSO, PEG, or glycerol, normally used to prevent drying of printed spots decrease binding and/or destroy spot morphology. The nature of the Protein Chip does not require that the printed spots remain solubilized for effective immobilization. Any amine containing buffers should be avoided in the spot solution.

Binding

Binding of proteins to the chip surface occurs through a thermo chemical reaction at room temperature (18-22°C). The saturated NaCl solution creates a 75% relative humidity environment that provides sufficient moisture for this reaction to proceed. If the printed slides are exposed to 100% relative humidity, the spots may enlarge or distort. To prepare the humidification chamber, add as much solid NaCl to water as needed to form a 1 cm deep slurry in the bottom of a plastic container with an airtight lid.

VII. Summary of Protein Chip protocol

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VI. Assay procedure

1. If required dilute specimen in Binding buffer.
2. Immediately apply 0,1 ml of specimen to the chip well.
3. Place chip in incubation oven at 37°C shaking for 30 minutes.
4. Discard the reacted liquid and add 250µL of Wash Buffer into each chip well, wait 30 sec and then discard the wash liquid; repeat this wash step 5 times. Invert the chip on paper towel and tap to adsorb residual liquid.
5. Dilute specific biotin conjugate to a final concentration of 0,1– 1µg/ml in Binding buffer. Note this is only a suggested range that may vary from assay to assay and therefore it should be determined by each final user.
6. Immediately apply 0,1 ml of biotin conjugate to the chip well.
7. Place chip in incubation oven at 37°C shaking for 15 minutes.
8. See point 4
11. Dilute Strep-AP 1/1000 using Binding Buffer and mix well, transfer 0,1 ml of the mixture into each chip well, and allow 15 minutes for reaction in incubation oven at 37°C shaking. Unused diluted Strep-AP can be stored at 2-8°C up to 3 months.
12. See point 4
13. Dilute NBT/BCIP 1/50 using Detection Buffer and mix well, transfer 0,1 ml of the mixture into each chip well, and allow 5 minutes for reaction at room temperature in the dark. Prepare just the volume needed because any unused diluted substrate should be used within 24 hours or discarded.
14. Discard the detection liquid, rinse the chip with 3 x 250 µl of bidistilled water. Invert the chip on paper towel to absorb residual liquid.
15. Dry the chip at 50°C in the oven for 5 minutes
16. Read the results of the developed pattern on the chip.

KIT CONTENT

Store between 2-8°C

No	Descriptions	Volume	Quantity
1	Binding Buffer	100 mL	1 bottle
2	Wash Buffer	250 mL	1 bottle
3	Strep-AP Conjugate	30 µL	1 vial
4	Blocking Buffer	100 mL	1 bottle
5	Detection Buffer	60 mL	1 bottle
6	NBT/BCIP	0,6 mL	1 vial
7	Printing Buffer	60 mL	1 bottle
8	Protein Chip	96 Rx	32 packs

REAGENTS

1	**Binding Buffer**	Specially formulated to facilitate the binding of specific proteins with analytic sample. Also used as a dilution buffer for conjugates.
2	**Wash Buffer**	Washing buffer to remove the unbound material. Ready to use solution.
3	**Strep-AP Conjugate**	Streptavidin conjugated with alkaline phosphatase as enzyme.
4	**Blocking Buffer**	Specifically formulated to reduce non-specific binding on Protein Chip surfaces.
5	**Detection Buffer**	Dilution buffer for NBT/BCIP.
6	**NBT/BCIP**	Chromogenic phosphatase substrates.
7	**Printing Buffer**	Spotting Buffer.
8	**Protein Chip**	1 x3 well activated chip

STORAGE CONDITIONS

Please store all reagents between +2°C and +8°C except Protein chip and Printing buffer that must be stored at room temperature (18-22°C).

PRECAUTION

- **All protocols in this manual shall be followed. Gloves shall be worn at all time. In case of contact with eyes, rinse eyes immediately with plenty of water.**
- **Do NOT touch the surface of the chip.**

MATERIALS REQUIRED BUT NOT PROVIDED

Instruments

Micropipettes	Vortex mixer	-20℃ Freezer
Hot plate	DR. Fast Spotter	Incubation oven
Microcentrifuge	DR. AiM Reader (optional)	Humidification chamber

Consumables

96 well plate	Tips	1.5 mL microcentrifuge tubes

PROTOCOLS

Notices :

- **Printing buffer may contain particulate matter when stored at +4°C, please warm up to 37°C for 25 minutes and leave at room temperature before use.**

I. Spotting protein dilution

Dilute protein to a final concentration of 0,1-1 mg/ml in Printing Buffer. Protein challenge and optimal buffer may vary from assay to assay and therefore it should be determined by each final user.

ATTENTION:

A minimal protein dilution of 1/2 into Printing Buffer is required, otherwise sample may not flow into the microfluidic channels of the Fast Spotter loading tray.

II. Spotting and binding

1. Remove the chip from the sealed package. Unused chip should be stored inside foil pouch with desiccant.
2. Print protein solution on activated chip following the DR Fast Spotter IFU.
3. Place printed chip in a storage box.
4. Set uncovered storage box in the humidification chamber.
5. Seal chamber and allow to incubate at room temperature. Overnight incubation has shown the best results. Alternatively incubate for 2 hours at 37°C in humid chamber.

III. Post coupling

1. Rinse the chip twice with Blocking Buffer. Use at least 0,25 ml per well.
2. Place the chip in a tray and block residual reactive groups using Blocking buffer for 1 hour at 37°C shaking. Use at least 0,25 ml per well.
3. Discard the blocking solution. Invert the chip on paper towel to absorb residual liquid.

If chip must be used the same day proceed to point V avoiding drying step.

IV. Drying

1. Dry the chip at 37°C in the oven for 30 minutes.
2. Put chip into sealed plastic bag with silica gel inside.

V. Specimen collection and preparation

Specimens showing particulate matter or turbidity should be centrifuged (relative centrifugal force of 1000-1200g for 5-15 min) before testing.
Before testing, totally thaw deep-frozen specimens, bring to room temperature (15-30°C), mix well and centrifuge (relative centrifugal force of 1000-1200g for 5-15 min) where appropriate.