ANTIBODY MICROARRAY PROTOCOL

1. INTRODUCTION

Overview

Both sides of NSB slides are coated with trillions of NanoCones™, cone-shaped organic chemical compounds. The apexes of NanoCones are functionalized with a NHS (N-hydroxyl succinimidyl) or aldehyde groups for directly attaching probe molecules with amino groups. NanoCones provide controlled regular spacing between the functional groups to minimize steric hindrance and enhance the kinetics. A judicious selection of the underlying layer results in the low non-specific binding. Microarrays printed on NSB slides boast high specificity/sensitivity with a low background signal. Currently, provided are the slides coated with two different sizes of NanoCones: NSB9 slide and NSB27 slide. NSB9 slides are more optimized for oligonucleotide DNA microarrays due to 3 to 4 nm of lateral spacing between the functional groups on the slide, while NSB27 slides with 6 to 7 nm of lateral spacing are more preferable for microarrays printed with cDNA, antibody, antigen, peptide, aptamer, and so on.

Handling and Storage Instructions

- The shelf-life of the packaged NSB slides is six months when they are stored in a desiccator at room temperature as received. Please check the expiry date labeled on the package as soon as you receive them.
- After opening the package, it is recommended that the slides be kept in a desiccator and used within one week for the optimal outcome.
- Use the slides in a clean environment, since dust particles may cause problems.
- Avoid direct contact with the surface while handling. Hold the edges of the slides and use only powder free gloves to minimize contamination and damage to the coating.
- Refer to manufacturer supplied Material Safety and Data Sheets (MSDS) for proper handling and disposal of all chemicals. MSDS are available upon request.

Limited Right of the Use

NSB slides are sold for research purposes only and are not intended for resale. This product should not be used in human diagnostics or for drug purposes.

Certain arrays and/or methods of preparation, analysis, or use may be covered by intellectual property rights in certain countries. Use of this product is recommended only for applications for which the user has a license under proprietary rights of the third parties or for technology for which a license is not required.

If you intend to use NSB slides for commercial purposes, please contact us.
2. ARRAY FABRICATION AND PRINTING

Solutions & Reagents Required:
- Spotting solution: 1X PBS (pH 8.5) including 0.1 mg/ml BSA, 0.01% Tween 20 and 0.5% glycerol. For convenience, 2X concentrated spotting solution can be made and added 1:1 ratio to your antibody solution.

1) Dilute antibodies in the spotting solution above. Antibody probe concentration ranging from 0.2 to 1.0 mg/ml can be used for printing, but 0.3 to 0.6 mg/ml concentration is more recommended to ensure sufficient protein loading and to enable reliable and consistent assay results. Because higher concentration of tween 20 and glycerol in your spotting solution increases printed spot size especially as a contact type of a microarrayer is used for printing, you should keep the concentration low if you want to fabricate a high density microarray.

2) Set up a microarrayer and print slides in accordance with the manufacturer’s or laboratory protocol. The printing environment should be dust free, kept at room temperature (20 - 25 °C) and at a relative humidity between 50 and 55 %.

3) Keep the printed arrays at 75 to 85 % relative humidity (in a humidity chamber or in the printing instrument) and room temperature (20 - 25 °C) for more than 5 hours (Overnight incubation is recommended.). The humidity chamber can be easily constructed by putting a cotton pad soaked with a saturated salt solution in a container with a lid such as a glass staining jar. Alternatively, a small glass dish holding the saturated salt solution can be placed in a big container such as a glass desiccator. Avoid direct contact between the salt solution and the arrays.

4) After incubation, you can use the slides immediately or if you want to store the printed slides, sequentially wash in PBST, PBS and ddH2O, and you can place the slides in a humidified slide box, seal and store at 4 °C for short period (less than 7 days), or just seal (no vacuum) and store at -20 for long time storage.
3. TARGET PREPARATION

Sera Treatment

Solutions & Reagents Required:
- 1X PBS at pH7.4
- PROT-1A Immunoaffinity Albumin and IgG Depletion Kit (Sigma)

1) Remove serum albumin by following the protocol of PROT-1A Immunoaffinity Albumin and IgG Depletion Kit (Sigma).
2) Measure the total protein concentration of depleted serum by BCA assay.

Fluorescent Labeling of Serum Proteins

Solutions & Reagents Required:
- PBST: 1X PBS (pH7.4) and 0.05% Tween-20
- 0.5 M Sodium Bicarbonate at pH 9.2 filtered through 0.2µm filter
- 1M Tris-HCl at pH 8.0
- Cy3 and Cy5 monoreactive dyes (40 nmole, GE Healthcare Code Number RPN5661) dissolved in 200 µl dehydrated DMSO

1) Place 2.5 µl depleted serum in a 1.5 ml tube (amount should be 2 - 4 mg/ml).
2) Combine 2.5 µl of 0.5 M Sodium Bicarbonate (pH 9.2) with 5 µl aliquot of Cy3 or Cy5 monoreactive dye. Then immediately transfer the dye mixture to the depleted serum sample.
3) Incubate in the dark at room temperature for 45 min.
4) Quench the reaction with 5 µl of 1 M Tris (pH 8) and incubate in the dark at room temperature for 15 min.
5) Combine Cy3 and Cy5 reactions (equal amount of labeled protein per sample) and add 450 µl of 1X PBST.
6) Transfer the sample solution to a Microcon 10 column (microcon YM-10, Millipore) and spin at 14000 X g for 30 min or until volume is reduced to about 100 µl.
7) Discard the flow-through.
8) Add 500 µl PBST to the column and spin at 14000 X g for 30 min or until volume is reduced to about 100 µl.
9) Repeat steps 7 and 8 two more times.
10) On the last spin make sure volumes are reduced to no less than 100 µl. If necessary, adjust the volume using PBST.
11) Invert the column into a fresh tube and elute the sample by spinning at 1000 X g for 2 min.
12) Proceed immediately to hybridization.
4. ANTIBODY ARRAY ASSAY

Slide Blocking Protocol

Solutions & Reagents Required:
All solutions should be made fresh unless otherwise indicated and all water used should be molecular biology grade. Fresh solution should be used for each rack.

- PBST: 1X PBS (pH7.4) and 0.05% Tween-20
- Blocking solution: 50 mM Ethanolamine in 50 mM sodium borate buffer (pH 8.0)

Once the protocol is started, it is important that the slides remain submerged in solution at all times.

1) Place printed slides in a slide rack.
2) Wash the slides in PBST by dunking vigorously 30 times.
3) Wash the slides in water by dunking vigorously 30 times.
4) Wash the slides in fresh water.
5) Place the slide rack in blocking solution and dunk vigorously 30 times.
6) Shake the solution containing the slide rack at 120 rpm for 2 hours.
7) Remove the slide rack from the blocking solution and place it in PBST solution.
8) Dunk vigorously 30 times in PBST.
9) Wash the slides in water two times.
10) Immediately dry slides by spinning at 1000 X g for 5 min at room temperature.
11) Proceed immediately to hybridization.

Array Hybridization

Solutions required:

- PBST: 1X PBS (pH7.4) and 0.05% Tween-20
- 1X PBS at pH 7.4

1) Load 100 µl of the previously labeled serum sample onto an Agilent gasket slide (4 microarrays/slide format, Agilent).

2) Carefully put the array slide on top of the gasket slide and assemble the Agilent hybridization chamber.
3) Place the assembled chamber into hybridization oven and rotate it at room temperature for 2 hours.
4) Remove the chamber from hybridization oven and immediately place it in a washing bath (ArrayIT, cat# HTW) filled with PBST. Proceed with the next slide and do this until all slides have been immersed in PBST.

5) Wash the slides for 5 minutes by stirring the PBST solution.

6) Wash again in fresh PBST solution for 5 minutes.

7) Remove the slides and place them in another washing bath filled with 1X PBS (pH7.4).

8) Wash the slides for 5 minutes by stirring the solution.

9) Wash again in fresh 1X PBS solution (pH7.4) for 5 minutes.

10) Dunk vigorously 30 times in 1X PBS solution.

11) Wash the slides twice in water.

12) Immediately dry slides by spinning at 1000 X g for 5 min at room temperature.

13) Scan slides immediately.

For technical support and the most current information about this and related products, please visit the web site, **www.nsbpostech.com** and contact us.