NHS Slide for Glycan Array Application

For Technical Assistance, Please Contact

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For Research Use Only

Introduction:

Z Biotech provides a full spectrum of high quality microarray slides for printing various microarrays, including protein microarrays, glycan microarrays, reverse-phase protein microarrays and small molecule microarrays. The NHS microarray slides contain highly reactive *n*-hydroxysuccinimide (NHS) ester surface, which forms covalent bonds with primary amines. The NHS slides offer high loading capacity and extremely low background for the immobilization of proteins (antibodies and antigens), amine-modified glycans and amine-modified small chemical molecules.

Storage and Handling:

Store at -20 °C prior to use! Allow package to equilibrate at room temperature (about 20 minutes) before opening. After opening, seal any unused slides in the reusable pouch with desiccant inside and refrigerate.

Avoid contact with the surface of the slides to minimize contamination and abrasion of the surface. Wear gloves and hold slide along with the edges.

Buffers Required:

- Blocking Buffer: NGBB (10106), NHS Glycan Blocking Buffer, 1X concentration (or 1% BSA in PBST (PBS with 0.05% Tween 20)).
- Assay Buffer: GAAB (10107), Glycan Array Assay Buffer, 1X concentration
- Wash Buffer: 20 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.05% Tween 20 (TBST)

Blocking:

1. Affix a assay adaptor onto slide surface;
2. Block the slide for at least 0.5 hour in Blocking Buffer at room temperature with gentle shaking at 80 rpm;

Binding Assay or Immunoassay:

1. Prepare samples (biotinylated or fluorescence-labeled) in GAAB Assay Buffer;
2. Remove the Blocking Buffer by vacuum suction or pipetting the liquid out of each assay chamber;
3. Immediately apply ~80 μl samples (prepared in step 1) to the appropriate chamber;
4. Cover the chamber windows with an adhesive seal to prevent evaporation during incubation;
5. Incubate the slide on the rotating shaker at 80 rpm for at least 1 hour;
6. After 1 hour incubation, remove the slide from shaker and remove cover;
7. Remove sample from each chamber by vacuum suction or pipetting out;

8. Wash each chamber by adding 100 μl Wash Buffer twice (5-minutes incubation on shaker for each wash);

For Biotinylated samples, following procedures below to stain the slides:

9. Add ~80 μl of Cy3 or Cy5 labeled streptavidin at 1 μg/ml concentration to each assay chamber;
10. Cover the chambers with an adhesive seal;
11. Incubate the slide on the rotating shaker at 80 rpm for at least 30 minutes;
12. Remove the sample from each chamber by vacuum suction or pipetting out;
13. Wash each chamber by adding 100 μl Wash Buffer;
14. Remove the 16-chamber adaptor and wash the slide in a Coplin jar of 100 ml Wash Buffer (5-minutes incubation on shaker);
15. Rinse the slide with Millipore water on shaker for 2 minutes;
16. Spin the slide in a slide centrifuge for 15 seconds;
17. Scan the slide with a fluorescent microarray scanner at the appropriate wavelengths and setting;
18. Save the scanning image and process the data with appropriate quantitation software.

For unlabeled antibody samples, following procedures below to stain the slides:

9. Add ~80 μl of Cy3 or Cy5 labeled secondary antibody at 1 μg/ml concentration to each assay chamber;
10. Cover the chambers with an adhesive seal;
11. Incubate the slide on the rotating shaker at 80 rpm for at least 30 minutes;
12. Remove the sample from each chamber by vacuum suction or pipette;
13. Wash each chamber by adding 100 μl Wash Buffer;
14. Remove the 16-chamber adaptor and wash the slides once in a Coplin jar of 100 ml Wash Buffer (5-minutes incubation on shaker);
15. Rinse the slide with Millipore water on shaker for 2 minutes;
16. Spin the slide in slide centrifuge for 15 seconds;
17. Scan the slide with a fluorescent microarray scanner at the appropriate wavelengths and setting;
18. Save the scanning image and process the data with appropriate quantitation software.

NHS Slide for Protein Array Applications

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Storage and Handling:

Store at -20 °C prior to use! Allow package to equilibrate to room temperature (in 20 minutes) before opening. After opening, seal any unused slides in the reusable pouch with desiccant inside and refrigerate.

Avoid contact with the surface of the slides to minimize contamination and abrasion of the surface. Wear gloves and hold slide along the edges.

Buffers Required:

- Printing Buffer: NEPPB, NHS/Epoxy Protein Printing Buffer, 2X concentration
- Blocking Buffer: 1% BSA in PBST (PBS with 0.05% (v/v) Tween 20, pH 7.4)
- Assay Buffer: PAAB, Protein Array Assay Buffer, 2X concentration
- Wash Buffer: 20 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.05% Tween 20

Array Printing and Incubation:

1. Print proteins at an appropriate concentration in 1X NEPPB Printing Buffer. A protein probe concentration ranging from 0.1 to 1 mg/ml is recommended to ensure sufficient protein loading and to enable reliable and consistent assay results;
2. Incubate printed slides in a humidified chamber (60% humidity) for 1 hour at room temperature.
3. Printed slide can be stored at -20ºC after printing and immobilization, but before washing and blocking.

Blocking:

1. Affix a 16-chamber assay adaptor onto slide surface;
2. Block the slide for 1 hour in Blocking Buffer at room temperature with static condition.

Binding Assay or Immunoassay:

1. Prepare target samples (i.e., binding proteins or primary antibodies) in an appropriate dilution factor in PAAB Assay Buffer;

2. Remove the Blocking Buffer liquid out of each assay chamber by vacuum suction or pipette;
3. Carefully apply 70 μl sample (prepared in step 1) to the appropriate chamber;
4. Cover the chambers with an adhesive seal to prevent evaporation during incubation;
5. Incubate slide on a rotating shaker at 60 rpm for 1 hour;
6. After 1 hour of incubation, remove slide from shaker and remove cover;
7. Remove sample from each chamber by vacuum suction or pipette;
8. Wash each chamber by adding 100 μl Wash Buffer twice (5-minutes incubation for each wash);

For biotinylated samples, following procedures below to stain the slides:

9. Add 70 μl of Cy3 or Cy5 labeled Streptavidin (or Avidin) at 0.5 μg/ml concentration to each assay chamber;
10. Cover the chambers with an adhesive seal;
11. Incubate the slide on the rotating shaker at 60 rpm for 30 minutes;
12. Remove the sample from each chamber by vacuum suction or pipette;
13. Wash each chamber by adding 100 μl Wash Buffer;
14. Remove the 16-chamber adaptor and wash the slides twice in a Coplin jar of 100 ml Wash Buffer (5-minutes incubation for each wash);
15. Rinse the slide with 100 ml 0.01x PBS and DI water respectively;
16. Spin the slide in slide centrifuge for 15 seconds;
17. Scan the slide with a fluorescent microarray scanner at the appropriate wavelengths and setting;
18. Save the scanning image and process the data with appropriate quantitation software.

For unlabeled antibody samples, following procedures below to stain the slides:

10. Add 70 μl of Cy3 or Cy5 labeled secondary antibody at 0.5 μg/ml concentration to each assay chamber;
11. Cover the chambers with an adhesive seal;
12. Incubate the slide on the rotating shaker at 60 rpm for 30 minutes;
13. Remove the sample from each chamber by vacuum suction or pipette;
14. Wash each chamber by adding 100 μl Wash Buffer;
15. Remove the 16-chamber adaptor and wash the slides twice in a Coplin jar of 100 ml Wash Buffer (5-minutes incubation for each wash);
16. Rinse the slide with 100 ml 0.01x PBS and DI water respectively;
17. Spin the slide in slide centrifuge for 15 seconds;
18. Scan the slide with a fluorescent microarray scanner at the appropriate wavelengths and setting;
19. Save the scanning image and process the data with appropriate quantitation software.