Protocol	
Multivalent Hydrazide Slides **10501-3**	Version 2.6
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For Technical Assistance, Please contact

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Introduction:

Z Biotech provides a full spectrum of high-quality microarray slides for printing glycan microarray. The hydrazide-functionalized substrate slides provide the only microarray slides on the market that are capable for fabricating glycan microarrays with glycans containing free reducing-end. Additionally, the hydrazide-functionalized slide also can be used for site-specific immobilization of proteins through the carbohydrate portion after $NaIO_4$ oxidation. The Hydrazide slides are highly recommended for glycomics, high-throughput glycan microarrays, and all other applications requiring premium, high quality microarray surface. For customer to obtain best microarray results, we also provide validated printing buffers, blocking buffer, and assay buffers for each type of microarray slides.

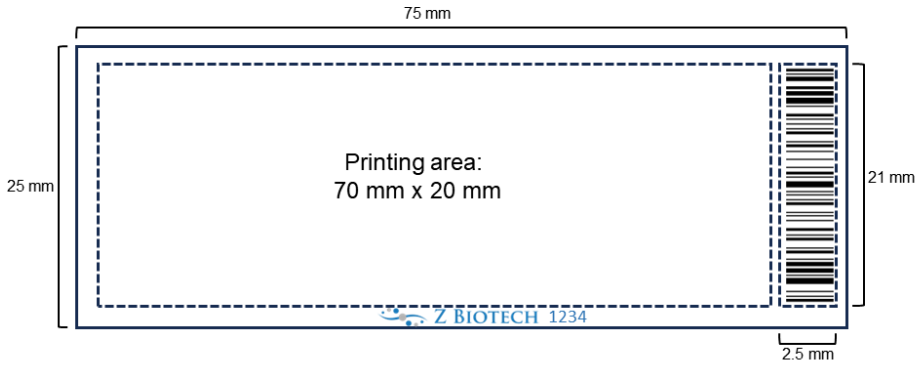
Storage and Handling:

Store at 4 °C prior to use! Allow package to equilibrate at room temperature (about 10 minutes) before opening. After opening, seal any unused slides in the reusable pouch 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.

Slides Coating Indication:

The slides are coated with the hydrazide chemistry on the side with the "Z Biotech" label and 4-digit number ID facing upward. The "Z Biotech" label is located on the bottom center from a landscape view. The number ID is consistent with the barcode ID on the bottom from a portrait view. Dimensions and array maps are shown below.



Buffers Required:

- Printing Buffer: HGPB, Hydrazide Glycan Printing Buffer, 2X concentration
- Blocking Buffer: Glycan Array Blocking Buffer (GABB, Item #10106)
- Glycan Array Assay Buffer (GAAB, Item #10107), add 1% BSA (10 mg/ml) if needed
- Wash Buffer: 20 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.05% Tween 20 (TBST)
- Laser fluorescence scanner (able to scan at the wavelength of your fluorophore)
- Coplin jar
- Adhesive slide cover film

Array Printing and Glycan Immobilization:

1. Prepare glycans at an appropriate concentration in 1X HGPB Printing Buffer. A glycan probe concentration ranging from 0.01 to 0.1 mM is recommended to ensure sufficient glycan loading and to enable reliable and consistent assay results;
    - The Hydrazide slides are developed for covalent attachment of glycans that have reducing-end. The coupling efficiency of the covalent chemistry depends on a number of factors, including pH, glycan print concentration, and the immobilization temperature.
    - The Hydrazide slides are compatible with all microarray printing or spotting methods, including contact printing and piezo or ink-jet technologies.
2. Print the glycan probe solution on Hydrazide slide in a humidified chamber (50-60% humidity).
3. Two options to enhance the glycan immobilization
    - Incubate the slide in a humidified chamber (50% humidity) at 50°C overnight;
    - Alternatively, place a humidified chamber containing printed slide in a microwave oven and perform microwave treatment. The results depend on model and power of the microwave.
4. Printed slides can be stored in dried environments before use.

Assay Protocol

<u>Preparation of assay samples:</u>

Prepare glycan-binding protein samples or secondary antibodies of interest in a centrifuge tube by diluting with the Glycan Array Assay Buffer. We recommend a range of 50 μg/ml to 0.1 μg/ml concentration for protein samples, although some experimentation may be required to establish the concentration that will provide the highest binding signals with the lowest background fluorescence. This is often accomplished by applying a different dilution of samples to different wells of the array. For the fluorescently labeled streptavidin, we recommend a concentration of 1 μg/mL. Calculate the volume of sample needed depending on how many slides and subarrays are to be assayed. We recommend using 100 μL sample per well for 16 subarray cassettes and 200 μL for 8 subarray cassettes to ensure full and even coverage of the printed area throughout incubation for every step of the assay. If necessary, the assay can be done successfully with a

minimal volume of 60 µL per well for 16 subarray cassettes and 80 µL for 8 subarray cassettes. We caution that using a minimal volume in the wells has an increased risk of the array drying out during the assay and may also cause unequal distribution of the sample across the arrayed surface which may result in signal variation. Please ensure each sample is homogeneous and thoroughly mixed.

Part 1 – Blocking

Handle the slide in a clean, dry environment. Use gloves and avoid touching the slide surface.

1. Let the arrayed slides equilibrate to room temperature (20-30 minutes) before opening the moisture barrier bag.
2. Add the blocking buffer to each subarray well.
3. Cover the wells with adhesive film to prevent evaporation and incubate slide on a shaker at 80 rpm for 30 min. Longer incubation time is acceptable, but not necessary.

Make sure the orbital shaker is completely flat. If the slide is sloped in any direction during incubation, it can cause variation in binding and detection.

Part 2 – Binding Assay

1. Unless the glycan-binding protein sample of interest is bacteria or cells, centrifuge samples briefly to avoid adding irrelevant particles to the array.
2. Remove the blocking buffer from each well by gently touching a pipette tip to the corner of the well, tipping the slide so that the sample pools to that corner, and pipetting off the buffer. Avoid touching the array surface. Have the replacement buffer ready before removing the old buffer to ensure the array does not dry out.
3. Wash the wells three times by adding GAAB to each well and shaking the array at 80 rpm for 5 min. Remove the buffer and repeat.
4. Immediately apply the glycan-binding protein sample of interest to each well. Avoid leaving air bubbles.
5. Seal the wells with adhesive film to prevent evaporation. If the sample is fluorescently labeled, cover it with aluminum foil to keep it in the dark. Incubate on the shaker for 1 hour at 80 rpm. If the samples can easily aggregate, shake them at a higher speed to prevent protein aggregation. Longer incubation time may increase the binding signal, especially for weakly binding samples.

Avoid allowing the slides to dry out at any point during the assay, especially during long incubation times. Make sure the adhesive film is sealed around each well.

If your glycan-binding protein samples are fluorescently labeled, go directly to Part 6 – Final Wash and Dry.

Part 3 – Wash

1. Remove buffer or sample from each well by gently touching a pipette tip to the corner of the well, tipping the slide so that the sample pools to that corner, and pipetting off the buffer. Avoid touching the array surface.
2. Immediately add GAAB to each well. Incubate on the shaker for 5 minutes at 80 rpm. Completely remove the buffer by pipette and repeat this step twice more. Avoid allowing the slide to dry out by having your next wash or sample ready before you remove the buffer.

If your glycan-binding sample is biotinylated, go directly to Part 5 – Fluorescent Staining.

Part 4 – Binding of Biotinylated Antibody (Sandwich Assay Format)

1. Unless the secondary biotinylated antibody sample is bacteria or cells, centrifuge samples briefly to avoid adding irrelevant particles to the array.
2. After removing the third GAAB wash, immediately add the secondary biotinylated antibody to each well. Seal the wells with adhesive film and incubate on the shaker for 1 hour at 80 rpm. Shaking at a faster speed can prevent protein aggregation. Longer incubation time is acceptable, but not necessary.
3. After incubation, repeat Part 3 – Wash.

Part 5 – Fluorescent Staining

1. Centrifuge fluorescent labeled streptavidin samples briefly to avoid adding irrelevant particles to the array.
2. After completely removing the third GAAB wash, immediately add the fluorescently labeled streptavidin sample. Seal the wells with adhesive film and shield the wells from light with aluminum foil. Incubate on the shaker at 80 rpm for 1 hour. Longer incubation time is acceptable, but not necessary.

Part 6 – Final Wash and Dry

1. Remove the sample from each well by gently touching a pipette tip to the corner of the well, tipping the slide so that the liquid pools to that corner, and pipetting off. Avoid touching the array surface.
2. Briefly rinse each well with GAAB.
3. Completely remove the buffer by pipette. Avoid touching the array surface. Repeat steps 2 and 3.
4. Disassemble the cassette from the slide. For the provided cassette, this can be done by holding the slide with one hand at the top and bottom edges and sliding out the cassette clips one by one with the other hand. If your provided cassette has metal clips, they can be removed by rotating the clip outwards from the bottom of the slide. When the clips have been removed, place the slide on the table and hold a small outer edge of the slide to the table as you gently peel the cassette off.
5. Immediately immerse the slide in a Coplin jar or beaker full of GAAB. Do not touch the surface of the array or allow the array surface to touch the sides of the beaker or jar.

6. Place the jar or beaker on a shaker at 80 rpm for 10 minutes.
7. Decant the buffer from the jar or beaker while holding the slide in place (only touch the edge of the slide) and then add sterile de-ionized water to immerse the slide.
8. Place the jar or beaker on the shaker at 80 rpm for 2 minutes.
9. Decant the water from the jar or beaker. Repeat once more with fresh de-ionized water.
10. Allow the slide to dry completely in a clean, dust-free environment before scanning.

Analysis

Scan the slide in a laser fluorescence scanner at the wavelength of emission for the fluorophore used. Adjust the laser power and PMT to obtain the highest possible signals without being saturated (saturated positive control signal is okay). Analyze data with microarray analysis software. If there is specific binding, the signal intensity should be higher than the background signal (the area where there are no printed spots). The fluorescent signal due to specific binding to your sample of interest should be dose-dependent with your sample dilution (unless the sample concentration range is too high and glycan-binding is saturated) and should have a positive binding signal after the signal from control assays has been subtracted. Our standard method of comparing signal intensities is to quantify the median signal intensity data and subtract the background intensity. Subtracting signals from negative control spots as well as the same spots on a negative control assay (assay with only detection antibodies and fluorophore) will give more accurate specific binding data.