Oligomannose Glycan Array

User Manual

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Introduction

Oligomannose glycans are present in various glycoproteins, and play a critical role in several biological processes. One essential function of oligomannoses is to facilitate the trafficking of lysosomal hydrolases by interacting with mannose-6-phosphate (Man-6-P) receptors, ensuring the correct delivery of lysosomal hydrolases to their destination, and ultimately contributing to the proper functioning of cells and the prevention of disease. Additionally, oligomannoses attached to glycoproteins within the endoplasmic reticulum are necessary for proper protein folding and assembly. If a protein is misfolded, oligomannose glycans activate the protein quality control system, leading to protein degradation, which maintains cellular homeostasis and prevents the accumulation of misfolded or aberrant proteins.

Apart from their role in cellular processes, oligomannose glycans also play a crucial role in immune recognition between hosts and microorganisms. Oligomannose glycans act as ligands for FimH, an adhesion protein located on the type 1 fimbriae of uropathogenic Escherichia coli, facilitating bacterial attachment and subsequent infection in human urinary epithelial cells. In contrast, oligomannose glycans act as pathogen-associated molecular patterns (PAMPs) in yeast and other microorganisms, and are recognized by a group of innate immune receptors known as C-type lectin receptors (CLRs), such as DC-SIGN, L-SIGN, Dectin-2, and Langerin. These CLRs are expressed on the surface of dendritic cells, macrophages, and other immune cells, and recognize oligomannose PAMPs to initiate immune responses. This is critical in shaping the innate immune response to microorganisms.

Although numerous oligomannose-binding proteins have been identified, their substrate specificity remains largely unexplored due to the lack of tools that facilitate relevant studies. To address this gap, Z Biotech has developed a robust oligomannose microarray platform that enables researchers to explore interactions between oligomannose glycans and various biological samples. The oligomannose array comprises 46 distinct oligomannose glycan structures, including linear, branched, high-mannose, and phosphorylated oligomannose glycans. These closely related isoforms with defined linkages offer a comprehensive group of glycans for studying mammalian and microbial mannose-binding proteins. The array system includes 8 or 16 identical subarrays, enabling the simultaneous analysis of multiple samples. The assay format is simple, requires only a small sample volume, and provides reliable glycan-binding information with high throughput.This manual provides a comprehensive guide for researchers to obtain clear results from the assay. We strongly recommend carefully reading through it before conducting your experiment.

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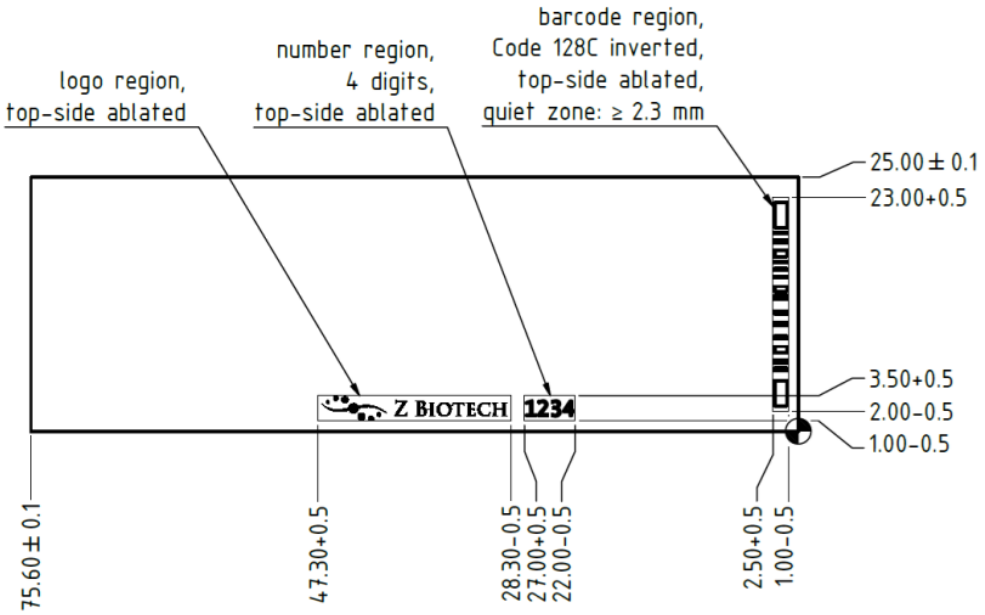
Handling and Storage

Store the bag of slides and any buffers in a 4°C refrigerator if they are to be assayed within 24 hours upon receipt. For a long-term storage, keep the bag of slides at -20°C. Avoid freezing and thawing multiple times. Purchased slides and buffers should be used within 6 months.

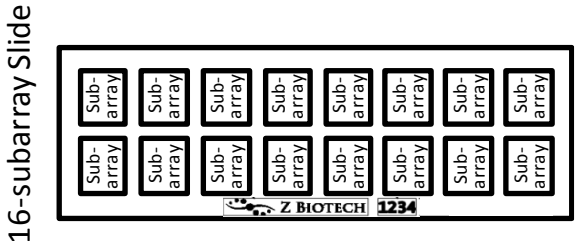
Allow the bag of slides to equilibrate to room temperature at least 20 minutes before opening. After opening, re-seal any unused slides in the moisture barrier bag with a desiccant inside and refreeze. Handle the slides in a dust-free environment. Wear gloves and hold the slides on the edges. When adding samples, do not touch the pipette tip to the array surface. When removing the sample, gently touch the pipette tip at the corner of the well of the cassette and tip the slide so that the sample pools to that corner. Avoid contact with the surface of the slides.

Array Map/Schematic

Oligomannose Glycan Array slides have 16 subarrays. Arrays are printed 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.



Array Map (16-subarray slides):



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OM5	OM5	OM5	OM5	OM6	OM6	OM6	OM6	OM7	OM7	OM7	OM7	OM8	OM8	OM8	OM8
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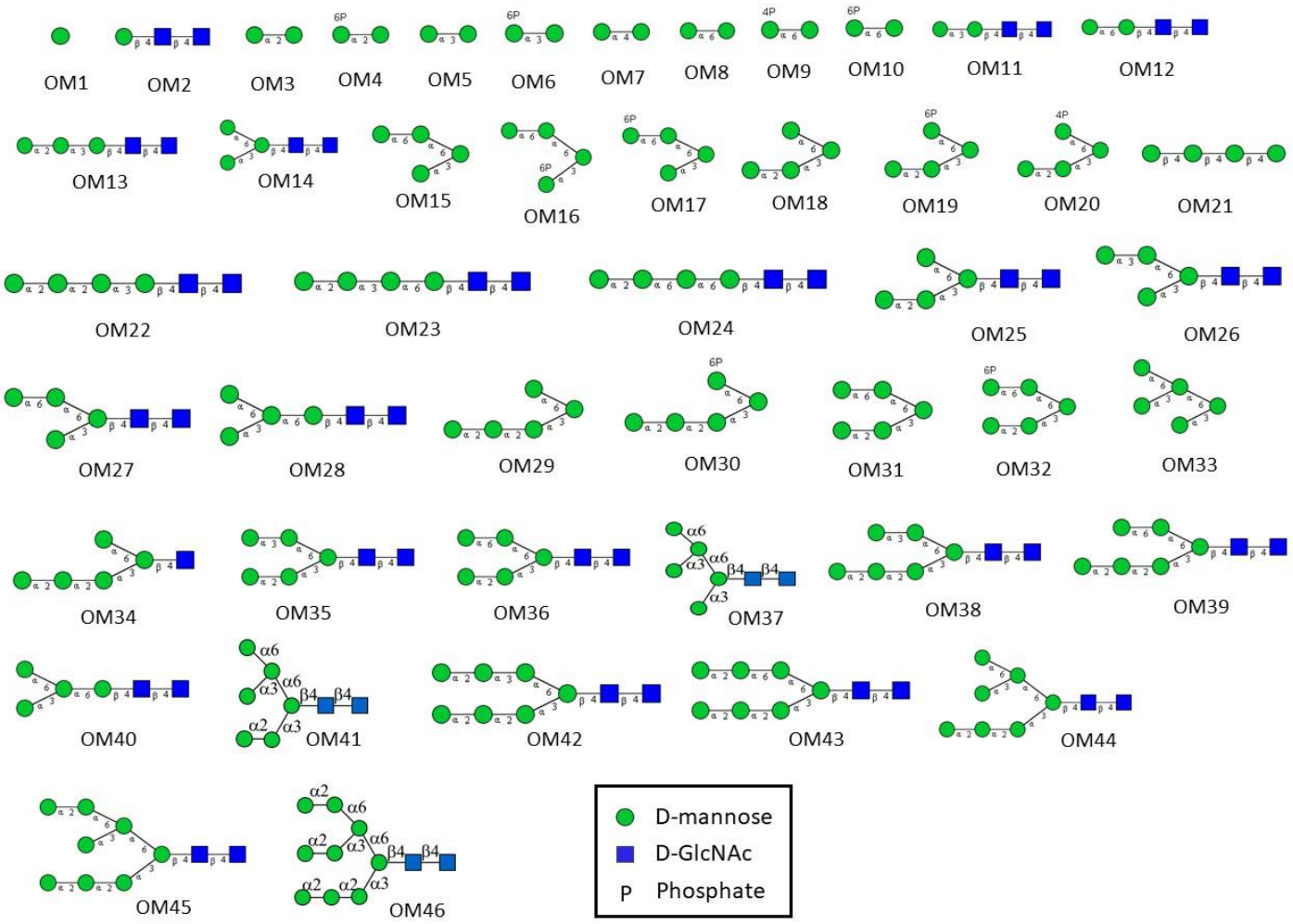
OM37	OM37	OM37	OM37	OM38	OM38	OM38	OM38	OM39	OM39	OM39	OM39	OM40	OM40	OM40	OM40
OM41	OM41	OM41	OM41	OM42	OM42	OM42	OM42	OM43	OM43	OM43	OM43	OM44	OM44	OM44	OM44
OM45	OM45	OM45	OM45	OM46	OM46	OM46	OM46	NC1	NC1	NC1	NC1	NC2	NC2	NC2	NC2
PC1	PC1	PC1	PC1	PC2	PC2	PC2	PC2	PC3	PC3	PC3	PC3	PC4	PC4	PC4	PC4
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	M	M	M	M

Oligomannose Glycan Identification List (SNFG Structures):



Oligomannose Glycan Identification List:

ID	Structure
OM1	Man
OM2	Manβ1-4GlcNAcβ1-4GlcNAc
OM3	Manα1-2Man
OM4	Man6Pα1-2Man
OM5	Manα1-3Man

OM6	Man6Pα1-3Man
OM7	Manα1-4Man
OM8	Manα1-6Man
OM9	Man4Pα1-6Man
OM10	Man6Pα1-6Man
OM11	Manα1-3Manβ1-4GlcNAcβ1-4GlcNAc
OM12	Manα1-6Manβ1-4GlcNAcβ1-4GlcNAc
OM13	Manα1-2Manα1-3Manβ1-4GlcNAcβ1-4GlcNAc
OM14	Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
OM15	Manα1-6Manα1-6(Manα1-3)Man
OM16	Manα1-6Manα1-6(Man6Pα1-3)Man
OM17	Man6Pα1-6Manα1-6(Manα1-3)Man
OM18	Manα1-2Manα1-3(Manα1-6)Man
OM19	Manα1-2Manα1-3(Man6Pα1-6)Man
OM20	Manα1-2Manα1-3(Man4Pα1-6)Man
OM21	Manβ1-4Manβ1-4Manβ1-4Man
OM22	Manα1-2Manα1-2Manα1-3Manβ1-4GlcNAcβ1-4GlcNAc
OM23	Manα1-2Manα1-3Manα1-6Manβ1-4GlcNAcβ1-4GlcNAc
OM24	Manα1-2Manα1-6Manα1-6Manβ1-4GlcNAcβ1-4GlcNAc
OM25	Manα1-2Manα1-3(Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc
OM26	Manα1-3Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
OM27	Manα1-6Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
OM28	Manα1-3(Manα1-6)Manα1-6Manβ1-4GlcNAcβ1-4GlcNAc
OM29	Manα1-2Manα1-2Manα1-3(Manα1-6)Man
OM30	Manα1-2Manα1-2Manα1-3(Man6Pα1-6)Man
OM31	Manα1-2Manα1-3(Manα1-6Manα1-6)Man

OM32	Manα1-2Manα1-3(Man6Pα1-6Manα1-6)Man
OM33	Manα1-6(Manα1-3)Manα1-6(Manα1-3)Man
OM34	Manα1-2Manα1-2Manα1-3(Manα1-6)Manβ1-4GlcNAc
OM35	Manα1-2Manα1-3(Manα1-3Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc
OM36	Manα1-2Manα1-3(Manα1-6Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc
OM37	Manα1-6(Manα1-3)Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
OM38	Manα1-2Manα1-2Manα1-3(Manα1-3Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc
OM39	Manα1-2Manα1-2Manα1-3(Manα1-6Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc
OM40	Manα1-2Manα1-3(Manα1-2Manα1-6)Manα1-6Manβ1-4GlcNAcβ1-4GlcNAc
OM41	Manα1-6(Manα1-3)Manα1-6(Manα1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
OM42	Manα1-2Manα1-2Manα1-3(Manα1-2Manα1-3Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc
OM43	Manα1-2Manα1-2Manα1-3(Manα1-2Manα1-6Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc
OM44	Manα1-6(Manα1-3)Manα1-6(Manα1-2Manα1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
OM45	Manα1-2Manα1-6(Manα1-3)Manα1-6(Manα1-2Manα1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
OM46	Manα1-2Manα1-6(Manα1-2Manα1-3)Manα1-6(Manα1-2Manα1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc

Controls

NC: Negative control, Print Buffer

PC1: Positive control 1, Biotinylated Mannose (0.01 mg/mL)

PC2: Positive control 2, Human IgG (0.1 mg/mL)

PC3: Positive control 3, Mouse IgG (0.1 mg/mL)

PC4: Positive control 4, Rabbit IgG (0.1 mg/mL)

Marker (M): Anti-human IgG, Cy3 (0.01 mg/mL) and anti-Human IgG, Alexa647 (0.01 mg/mL)

Materials Required

- Oligomannose Glycan Array slide
- 8-subarray cassettes
- Glycan Array Blocking Buffer (GABB, Item #10106), add 1% BSA (10 mg/ml) if needed
- Glycan Array Assay Buffer (GAAB, Item #10107), add 1% BSA (10 mg/ml) if needed
- Laser fluorescence scanner (able to scan at the wavelength of your fluorophore)

- Coplin jar
- Adhesive slide cover film

Preparation of assay samples:

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.

Assay Protocol

Considerations Before Starting the Experiment

1. **Preparation of Buffers:**
   - Ensure that Glycan Array Blocking Buffer (GABB) and Glycan Array Assay Buffer (GAAB) are ready for glycan microarray analysis.
2. **BSA Addition:**
   - If needed, add BSA to GAAB or LAAB to reduce non-specific binding.
   - Prepare a 1% BSA assay buffer by adding BSA to GAAB or LAAB and filter through a 0.2 μm PVDF membrane filter.
3. **Avoiding Dryness:**
   - The array surface is extremely sensitive to dryness. Ensure the array does not dry at any point during the assay.
   - Avoid handling multiple subarrays simultaneously to prevent drying out.
4. **Array Formats and Volumes:**
   - Common array formats: 8, 16, or 24 subarrays.
     - For 8-subarray format: Use 200 μL per subarray.
     - For 16-subarray format: Use 100 μL per subarray.
     - For 24-subarray format: Use 50-80 μL per subarray.
   - Minimal volumes: 60 μL per well for 16-subarray cassettes and 80 μL for 8-subarray cassettes.
   - Caution: Using minimal volumes increases the risk of drying out and may cause signal variation. Ensure samples are homogeneous and thoroughly mixed.
5. **Sample Preparation:**
   - Dilute glycan-binding protein samples or secondary antibodies in Glycan Array Assay Buffer.
   - Recommended concentration range for protein samples: 50 μg/mL to 0.1 μg/mL. Experiment to find the optimal concentration for highest binding signals with the lowest background.
   - For fluorescently labeled streptavidin, use a concentration of 0.2 μg/mL.
6. **Storage of Microarray Slides and Buffers:**
   - Store microarray slides and buffers at 4°C if assayed within 24 hours of receipt.
   - For long-term storage, keep microarray slides at -20°C. Avoid multiple freeze-thaw cycles.

- Use slides and buffers within 12 months. Allow slides to equilibrate to room temperature for at least 20 minutes before opening.
- After opening, reseal unused slides in a moisture barrier bag with a desiccant and refreeze. Handle slides in a dust-free environment, wearing gloves and holding slides by the edges.
- When adding samples, avoid touching the pipette tip to the array surface. When removing samples, gently touch the pipette tip to the corner of the well and tip the slide.

Analyzing Biological Samples with Glycan Microarray

Part 1: Blocking

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

1. Let the microarray slide equilibrate to room temperature (20-30 minutes) before opening the moisture barrier bag.
2. Assemble the slide into a hybridization chamber device.
3. Add the Glycan Array Blocking Buffer (GABB) to each subarray well of the assembled hybridization chamber device:
    - 100 µL for each subarray of a 16-subarray chamber device
    - 200 µL for each subarray of an 8-subarray chamber device
4. Cover the hybridization chamber device with adhesive film to prevent evaporation and incubate the slide on a shaker at 80 rpm for 30 minutes. Ensure the orbital shaker is completely flat to avoid variations in binding and detection. Longer incubation times are acceptable but not necessary.
5. After 30 minutes, add 100 µL of Glycan Array Assay Buffer (GAAB) to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device). Aspirate the liquid out from each well, ensuring that some liquid remains to cover the surface.
6. Add another 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device). Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
7. After incubation, aspirate all the liquid out from each well. Then add 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device). Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
8. After incubation, aspirate all the liquid out from each well. Then add 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device). Incubate at room temperature for 5 minutes at 80 rpm on a shaker.

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. We recommend doing this one subarray at a time, not handling multiple subarrays simultaneously to avoid drying out the microarray slide surface.
3. Immediately apply the glycan-binding protein sample of interest to each well.
4. 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.
5. Incubate on the shaker for 1 hour at 80 rpm. Longer incubation times 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.
6. If your glycan-binding protein samples are fluorescently labeled, go directly to Part 6 – Final Wash and Dry.

Part 3: Wash

We recommend doing the following procedure using a multi-channel pipette.

1. **Initial Wash:**
    - After incubating the samples for 1 hour at room temperature, use a multi-channel pipette to add 100 µL of Glycan Array Assay Buffer (GAAB) to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device).
    - Aspirate the liquid out using a multi-channel aspirating needle device from each well, ensuring not all the liquid is aspirated out, leaving enough liquid to cover the surface.
    - Repeat the addition and aspiration of GAAB three times. For each repetition, add 100 µL (or 200 µL for an 8-subarray device) of GAAB, aspirate, and ensure some liquid remains to cover the surface. Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
2. **Subsequent Washes:**
    - After the initial wash and incubation, aspirate all the liquid out from each well using a multi-channel aspirating needle device.
    - Add 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device).
    - Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
3. **Final Wash:**
    - After the second incubation, aspirate all the liquid out from each well using a multi-channel aspirating needle device.
    - Add 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device).
    - Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
4. **Proceed to the Next Step:**
    - If your glycan-binding sample is biotinylated, go directly to Part 5 – Fluorescent Staining.

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

1. **Adding Secondary Antibody:**
    - After removing all the GAAB using a multi-channel aspirating needle device, immediately add the secondary biotinylated antibody to each well using a multi-channel pipette.
2. **Incubation:**
    - Seal the wells with adhesive film and incubate on the shaker for 1 hour at 80 rpm. Longer incubation times are acceptable but not necessary.

Part 5: Fluorescent Staining

1. **Adding Streptavidin:**
    - After completely removing the GAAB using a multi-channel aspirating needle device, immediately add the fluorescently labeled streptavidin sample using a multi-channel pipette.
2. **Incubation:**
    - 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 times are acceptable but not necessary.

Part 6: Final Wash and Dry

1. **Initial Wash:**
    - After incubating the secondary antibody or streptavidin for 1 hour at room temperature, use a multi-channel pipette to add 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device).
    - Aspirate the liquid out using a multi-channel aspirating needle device from each well, ensuring not all the liquid is aspirated out, leaving enough liquid to cover the surface.
    - Repeat the addition and aspiration of GAAB three times. For each repetition, add 100 µL (or 200 µL for an 8-subarray device) of GAAB, aspirate, and ensure some liquid remains to cover the surface. Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
2. **Subsequent Washes:**
    - After the initial wash and incubation, aspirate all the liquid out from each well using a multi-channel aspirating needle device.
    - Add 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device).
    - Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
3. **Final Wash:**
    - After the second incubation, aspirate all the liquid out from each well using a multi-channel aspirating needle device.
    - Add 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device).
    - Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
4. **Disassembling the Hybridization Chamber:**
    - Disassemble the hybridization chamber device 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. **Immersing the Slide:**
    - Immediately immerse the slide in a Coplin jar 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. Place the jar or beaker on a shaker at 80 rpm for 10 minutes.
6. **Rinsing with Water:**
    - Decant the buffer from the jar while holding the slide in place (only touch the edge of the slide) and then add sterile de-ionized water to immerse the slide. Place the jar on the shaker at 80 rpm for 5 minutes.
7. **Repeat Rinsing:**
    - Decant the water from the jar. Repeat once more with fresh de-ionized water.
8. **Drying the Slide:**
    - Allow the slide to dry by using a microarray slide centrifuge completely in a clean, dust-free environment before scanning.

Part 7: Data Acquisition and Analysis

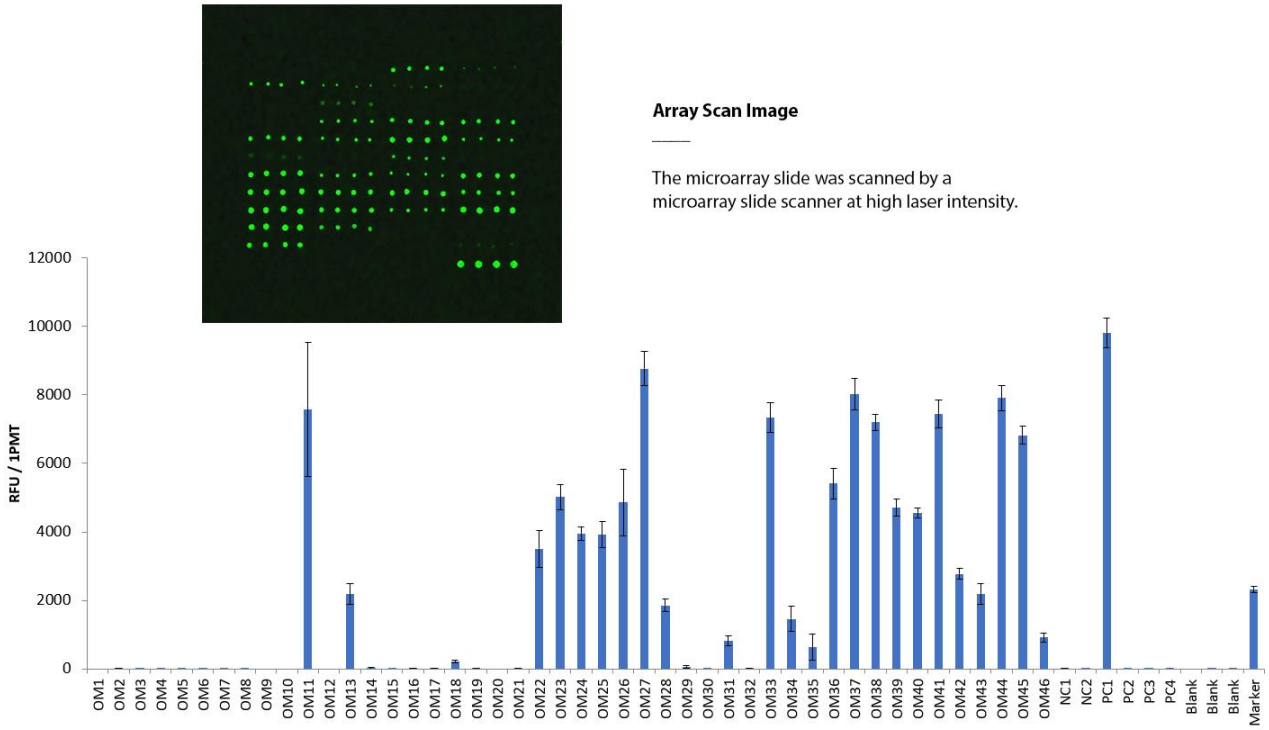
1. **Scanning the Slide:**
    - Scan the slide in a laser fluorescence scanner at the wavelength of emission for the fluorophore used. Adjust the laser power and PMT to obtain the highest possible signals without being saturated (saturated positive control signal is okay).
2. **Analyzing Data:**
    - 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.

3. **Quantifying Signal Intensities:**
    - 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.
4. **Interpreting Control Signals:**
    - **Negative Control (Print Buffer):** The negative control should produce a signal close to the intensity of the background. Since there is no binding involved with the negative control, any other signals around the negative control's intensity are also not binding.
    - **Marker:** The array marker should show a fluorescence signal regardless of the assay. It is there primarily to aid with the orientation of the array map during analysis.
    - **Biotinylated Mannose (PC1):** This positive control will bind directly to the fluorescent-labeled streptavidin. If your glycan-binding protein sample is already fluorescently labeled, or in any case where the addition of fluorescently labeled streptavidin to the array was not performed (Part 5 – Fluorescent Staining), this positive control will not be reactive.
    - **IgG (PC2, PC3, PC4):** IgG is an antibody found in the blood that is a primary component of humoral immunity. If the glycan-binding or secondary antibody sample is an anti-IgG from a human, rabbit, or mouse, it should bind to the respective IgG control.

Typical Binding Assay Result from the Oligomannose Glycan Array

The oligomannose glycan array was probed with biotinylated Galanthus nivalis lectin (GNA) at a 5 µg/mL concentration, followed by streptavidin labeled with Cy3. The array was scanned using a microarray scanner at a wavelength of 532 nm. The positive control (PC1) exhibited expected binding signals, and GNA demonstrated differential interactions with various oligomannose glycans. These results provide evidence of the array's functionality in probing oligomannose-binding proteins and studying their interactions with oligomannose glycans.



Troubleshooting

Condition	Possible Causes	Potential Solutions
High Background	• Concentration of glycan-binding protein samples is too high • Concentration of fluorescent samples is too high • Arrays are not thoroughly washed • Slide drying out during assay • Excessive particles in the samples due to sample aggregation, dust, etc.	• Use a lower concentration range of samples. Consider a wider range if you are unsure where the detection limit is. • Apply longer times for washing steps and use a higher shaking rate • Make sure wash buffer and sample is completely removed before the next step • Make sure adhesive film fully seals the wells to avoid evaporation • Centrifuge the samples prior to assay to avoid adding irrelevant particles • If you think that the protein is aggregating during incubation, try shaking at a higher speed
Signal Variation	• Slide drying out during assay. • Binding samples are not equally distributed in the wells • Glycan-binding protein aggregation during incubation • Bubbles during incubation	• Make sure wells are sealed to prevent evaporation during incubation. • Apply a larger volume of sample to each well to ensure equal distribution • Use a higher shaking rate during incubation • Make sure samples are homogeneous, mixed thoroughly, and do not leave bubbles on the array surface
Unexpected Binding	• Cross contamination between wells or other sources • Sample contamination	• Make sure to use sterilized pipette tips and tubes used for sample application and preparation • Ensure cassette is pressed firmly to the slide so that there are no gaps to allow leaking between wells • Be careful not to cross contaminate samples when applying to the wells, even during wash steps