Glycosaminoglycan Microarray User Manual

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For Research Use Only

Introduction

The pathogenesis of Alzheimer's Disease involves ubiquitous, linear polysaccharide molecules called glycosaminoglycans (GAGs) in the genesis of senile plaques and neuronal uptake of toxic tau protein aggregates. GAG interactions are largely electrostatic, and the GAG heparin – having the highest negative charge density of any known biological molecule – promotes tau protein aggregation by affecting its conformation and interprotein electrostatic repulsion. These protein aggregates can then bind to GAGs on neurons to gain entry to the cell. GAGs are also involved in many other biological processes from skin aging to tumor progression. Characterizing their roles, concentrations and configurations in relation to physiological stimuli could provide new approaches to disease prevention and regression.

Glycosaminoglycans are highly heterogeneous due to their varying conformations, molecular mass, and electrostatic potential, and their specific composition is determined by cell type. Their expression has been shown to change with age, ultraviolet radiation exposure, and at sites of injury, diseased tissues, and tumor growth. Further studies using new glycomic technologies are needed to help understand the presence of certain GAGs and their roles.

This Gycosaminoglycan Microarray compiles several varieties of GAGs in varying lengths, degrees of sulfation, and disaccharide sequences to aid in the efficient study of the functions and specific interactions of GAGs. Researchers can use this array to test molecules designed as inhibitory agents or disease, scar or injury-targeting therapeutics. This array can be used to investigate the GAG-binding specificity of viruses or VLPs, or determine the presence of specific GAG binders in sera, cerebrospinal fluid, or other biological samples.

This manual is provided as a comprehensive guide to help the researcher acquire clear results from the assay. Please read through carefully before starting your experiment.

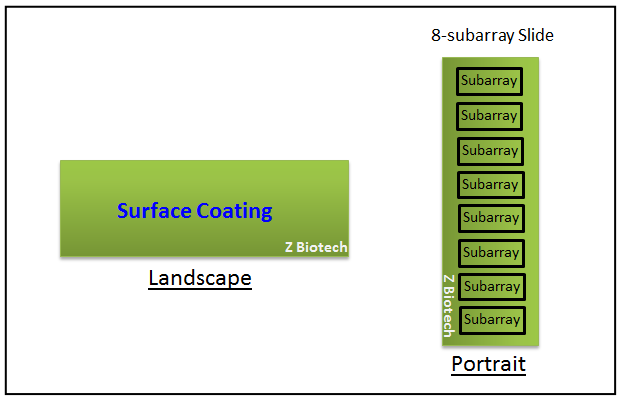
Handling and Storage

Store the bag of slides and any buffers in a 4°C refrigerator if they are to be assayed within 3 weeks upon receipt. For 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.

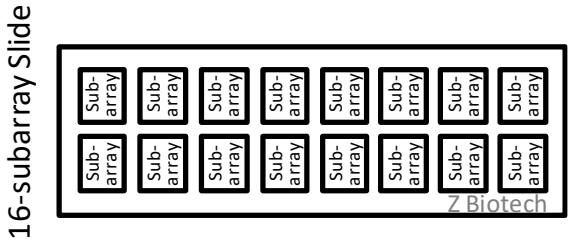
Allow the bag of slides to equilibrate to room temperature at least 20 minutes before opening. After opening, re-seal any unused slides in the moisture barrier bag with a desiccant inside and refreeze.

Array Map/Schematic

Glycosaminoglycan Microarray slides have either 8 or 16 subarrays. Arrays are printed on the side with the "Z Biotech" label facing upward. The "Z Biotech" label is located on the bottom right corner from a landscape point of view, or the bottom left corner in a portrait point of view (see image on right). The orientation of the printed array is consistent with the portrait slide orientation. Dimensions and array map are shown:

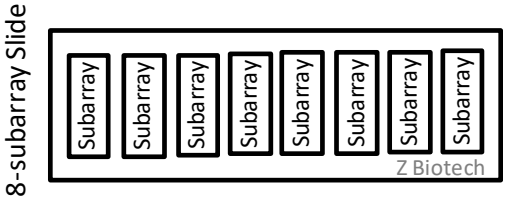


Array Map (16-subarray slides):



	1	2	3	4	5	6	7	8	9	10	11	12	13
1	MARKER	GAG1	GAG1	GAG1	GAG1	GAG2	GAG2	GAG2	GAG2	GAG3	GAG3	GAG3	GAG3
2	MARKER	GAG4	GAG4	GAG4	GAG4	GAG5	GAG5	GAG5	GAG5	GAG6	GAG6	GAG6	GAG6
3		GAG7	GAG7	GAG7	GAG7	GAG8	GAG8	GAG8	GAG8	GAG9	GAG9	GAG9	GAG9
4		GAG10	GAG10	GAG10	GAG10	GAG11	GAG11	GAG11	GAG11	GAG12	GAG12	GAG12	GAG12
5		GAG13	GAG13	GAG13	GAG13	GAG14	GAG14	GAG14	GAG14	GAG15	GAG15	GAG15	GAG15
6		GAG16	GAG16	GAG16	GAG16	GAG17	GAG17	GAG17	GAG17	GAG18	GAG18	GAG18	GAG18
7		GAG19	GAG19	GAG19	GAG19	GAG20	GAG20	GAG20	GAG20	GAG21	GAG21	GAG21	GAG21
8		GAG22	GAG22	GAG22	GAG22	GAG23	GAG23	GAG23	GAG23	GAG24	GAG24	GAG24	GAG24
9		GAG25	GAG25	GAG25	GAG25	GAG26	GAG26	GAG26	GAG26	GAG27	GAG27	GAG27	GAG27
10		GAG28	GAG28	GAG28	GAG28	GAG29	GAG29	GAG29	GAG29	GAG30	GAG30	GAG30	GAG30
11		GAG31	GAG31	GAG31	GAG31	GAG32	GAG32	GAG32	GAG32	GAG33	GAG33	GAG33	GAG33
12	MARKER	GAG34	GAG34	GAG34	GAG34	NC	NC	NC	NC	PC1	PC1	PC1	PC1
13	MARKER	PC2	PC2	PC2	PC2	PC3	PC3	PC3	PC3	PC4	PC4	PC4	PC4

Array Map (8-subarray slides):



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	GAG1	GAG1	GAG1	GAG1	GAG1	GAG1	GAG2	GAG2	GAG2	GAG2	GAG2	GAG2	GAG3	GAG3	GAG3	GAG3	GAG3	GAG3	GAG4	GAG4	GAG4	GAG4	GAG4	GAG4
2	GAG5	GAG5	GAG5	GAG5	GAG5	GAG5	GAG6	GAG6	GAG6	GAG6	GAG6	GAG6	GAG7	GAG7	GAG7	GAG7	GAG7	GAG7	GAG8	GAG8	GAG8	GAG8	GAG8	GAG8
3	GAG9	GAG9	GAG9	GAG9	GAG9	GAG9	GAG10	GAG10	GAG10	GAG10	GAG10	GAG10	GAG11	GAG11	GAG11	GAG11	GAG11	GAG11	GAG12	GAG12	GAG12	GAG12	GAG12	GAG12
4	GAG13	GAG13	GAG13	GAG13	GAG13	GAG13	GAG14	GAG14	GAG14	GAG14	GAG14	GAG14	GAG15	GAG15	GAG15	GAG15	GAG15	GAG15	GAG16	GAG16	GAG16	GAG16	GAG16	GAG16
5	GAG17	GAG17	GAG17	GAG17	GAG17	GAG17	GAG18	GAG18	GAG18	GAG18	GAG18	GAG18	GAG19	GAG19	GAG19	GAG19	GAG19	GAG19	GAG20	GAG20	GAG20	GAG20	GAG20	GAG20
6	GAG21	GAG21	GAG21	GAG21	GAG21	GAG21	GAG22	GAG22	GAG22	GAG22	GAG22	GAG22	GAG23	GAG23	GAG23	GAG23	GAG23	GAG23	GAG24	GAG24	GAG24	GAG24	GAG24	GAG24
7	GAG25	GAG25	GAG25	GAG25	GAG25	GAG25	GAG26	GAG26	GAG26	GAG26	GAG26	GAG26	GAG27	GAG27	GAG27	GAG27	GAG27	GAG27	GAG28	GAG28	GAG28	GAG28	GAG28	GAG28
8	GAG29	GAG29	GAG29	GAG29	GAG29	GAG29	GAG30	GAG30	GAG30	GAG30	GAG30	GAG30	GAG31	GAG31	GAG31	GAG31	GAG31	GAG31	GAG32	GAG32	GAG32	GAG32	GAG32	GAG32
9	GAG33	GAG33	GAG33	GAG33	GAG33	GAG33	GAG34	GAG34	GAG34	GAG34	GAG34	GAG34	NC	NC	NC	NC	NC	NC	PC1	PC1	PC1	PC1	PC1	PC1
10	PC2	PC2	PC2	PC2	PC2	PC2	PC3	PC3	PC3	PC3	PC3	PC3	PC4	PC4	PC4	PC4	PC4	PC4	Marker	Marker	Marker	Marker	Marker	Marker

Glycosaminoglycan Identification List:

ID	Name	Structure and Molecular Weight
GAG1	Hyaluronic Acid dp10 (HA10)	ΔGlcAβ1,3 [GlcNAcβ1,4 GlcAβ1,3]$_4$ GlcNAc, Mw 1,950 Da
GAG2	Hyaluronic Acid dp12 (HA12)	ΔGlcAβ1,3 [GlcNAcβ1,4 GlcAβ1,3]$_5$ GlcNAc, Mw 2,350 Da
GAG3	Hyaluronic Acid dp14 (HA14)	ΔGlcAβ1,3 [GlcNAcβ1,4 GlcAβ1,3]$_6$ GlcNAc, Mw 2,700 Da
GAG4	Hyaluronic Acid dp16 (HA16)	ΔGlcAβ1,3 [GlcNAcβ1,4 GlcAβ1,3]$_7$ GlcNAc, Mw 3,150 Da
GAG5	Hyaluronic Acid dp18 (HA18)	ΔGlcAβ1,3 [GlcNAcβ1,4 GlcAβ1,3]$_8$ GlcNAc, Mw 3,650 Da
GAG6	Hyaluronic Acid dp20 (HA20)	ΔGlcAβ1,3 [GlcNAcβ1,4 GlcAβ1,3]$_9$ GlcNAc, Mw 3,900 Da
GAG7	Hyaluronic Acid Polymer (HA93)	ΔGlcAβ1,3 [GlcNAcβ1,4 GlcAβ1,3]$_n$ GlcNAc, Mw 93 kDa
GAG8	Heparin dp10 (H10)	ΔIdoUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_4$, Mw 3,000
GAG9	Heparin dp12 (H12)	ΔIdoUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_5$, Mw 3,550
GAG10	Heparin dp14 (H14)	ΔIdoUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_6$, Mw 4,100
GAG11	Heparin dp16 (H16)	ΔIdoUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_7$, Mw 4,650
GAG12	Heparin dp18 (H18)	ΔIdoUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_8$, Mw 5,200
GAG13	Heparin dp20 (H20)	ΔIdoUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_9$, Mw 5,750
GAG14	Heparin dp22 (H22)	ΔIdoUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_{10}$, Mw 6,300
GAG15	Heparin dp24 (H24)	ΔIdoUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_{11}$, Mw 6,850
GAG16	Heparin dp30 (H30)	ΔIdoUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_{14}$, Mw 9,000
GAG17	Chondroitin Sulphate Oligosaccharide dp10 (CSO10)	ΔGlcAβ1,3 [GalNAc,6S or 4S - GlcA]$_4$ - GalNAc,6S or 4S, Mw 2,480
GAG18	Chondroitin Sulphate Oligosaccharide dp12 (CSO12)	ΔGlcAβ1,3 [GalNAc,6S or 4S - GlcA]$_5$ - GalNAc,6S or 4S, Mw 2,976
GAG19	Chondroitin Sulphate Oligosaccharide dp14 (CSO14)	ΔGlcAβ1,3 [GalNAc,6S or 4S - GlcA]$_6$ - GalNAc,6S or 4S, Mw 3,472
GAG20	Chondroitin Sulphate Oligosaccharide dp16 (CSO16)	ΔGlcAβ1,3 [GalNAc,6S or 4S - GlcA]$_7$ - GalNAc,6S or 4S, Mw 3,968
GAG21	Chondroitin Sulphate Oligosaccharide dp18 (CSO18)	ΔGlcAβ1,3 [GalNAc,6S or 4S - GlcA]$_8$ - GalNAc,6S or 4S, Mw 4,464
GAG22	Chondroitin Sulphate Oligosaccharide dp20 (CSO20)	ΔGlcAβ1,3 [GalNAc,6S or 4S - GlcA]$_9$ - GalNAc,6S or 4S, Mw 4,960
GAG23	Chondroitin Sulphate D Oligosaccharide dp10 (CSDO10)	ΔGlcAβ1,3 [GalNAc,6S or 4S – GlcA +/- 2S]$_4$ – GalNAc,6S, Mw 2,480
GAG24	Chondroitin Sulphate D Oligosaccharide dp12 (CSDO12)	ΔGlcAβ1,3 [GalNAc,6S or 4S – GlcA +/- 2S]$_5$ – GalNAc,6S, Mw 2,976
GAG25	Chondroitin Sulphate D Oligosaccharide dp14 (CSDO14)	ΔGlcAβ1,3 [GalNAc,6S or 4S – GlcA +/- 2S]$_6$ – GalNAc,6S, Mw 3,472
GAG26	Chondroitin Sulphate D Oligosaccharide dp16 (CSDO16)	ΔGlcAβ1,3 [GalNAc,6S or 4S – GlcA +/- 2S]$_7$ – GalNAc,6S, Mw 3,968
GAG27	Chondroitin Sulphate D Oligosaccharide dp18 (CSDO18)	ΔGlcAβ1,3 [GalNAc,6S or 4S – GlcA +/- 2S]$_8$ – GalNAc,6S, Mw 4,464
GAG28	Chondroitin Sulphate D Oligosaccharide dp20 (CSDO20)	ΔGlcAβ1,3 [GalNAc,6S or 4S – GlcA +/- 2S]$_9$ – GalNAc,6S, Mw 4,960
GAG29	Dermatan Sulphate dp10 (DS10)	ΔIdoAβ1,3 - GalNAc,4S – (IdoA – GalNAc,4S)$_4$, Mw 2,480
GAG30	Dermatan Sulphate dp12 (DS12)	ΔIdoAβ1,3 - GalNAc,4S – (IdoA – GalNAc,4S)$_5$, Mw 2,976
GAG31	Dermatan Sulphate dp14 (DS14)	ΔIdoAβ1,3 - GalNAc,4S – (IdoA – GalNAc,4S)$_6$, Mw 3,472
GAG32	Dermatan Sulphate dp16 (DS16)	ΔIdoAβ1,3 - GalNAc,4S – (IdoA – GalNAc,4S)$_7$, Mw 3,968
GAG33	Dermatan Sulphate dp18 (DS18)	ΔIdoAβ1,3 - GalNAc,4S – (IdoA – GalNAc,4S)$_8$, Mw 4,464
GAG34	Dermatan Sulphate dp20 (DS20)	ΔIdoAβ1,3 - GalNAc,4S – (IdoA – GalNAc,4S)$_9$, Mw 4,960

Controls

NC: Negative control, Print Buffer

PC1: Positive control 1, a biotinylated probe (0.01 mg/ml)

PC2: Postitive control 2, Human IgG (0.1 mg/ml)

PC3: Postitive control 3, Mouse IgG (0.1 mg/ml)

PC4: Postitive control 4, Rabbit IgG (0.1 mg/ml)

Array Marker: Anti-Human IgG, Cy3 (0.01 mg/ml) and anti-Human IgG, Alexa555 (0.01 mg/ml)

Materials Required

- Arrayed glass slide
- 16 or 8 subarray cassettes
- Blocking Buffer: NHS Glycan Blocking Buffer (NGBB Item #10106)
- Glycan Array Assay Buffer (GAAB Item #10107)
- Wash Buffer: 20 mM Tris-HCl, 150 mM NaCl, 0.1% Tween 20, pH 7.6
- Glycan-binding samples of interest
- 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 samples or detection antibodies in a centrifuge tube by diluting with the GAAB buffer. For the fluorescently labelled streptavidin we recommend a concentration of 1 μg/mL. For detection antibodies, we suggest a concentration of around 1-10 μg/ml. A range of 100 μg/ml to 0.1 μg/ml concentration for glycan-binding samples works, although some experimentation may be required to establish the concentration that will provide the highest binding signals with the lowest background fluorescence. This can be accomplished by applying a different dilution of samples to different wells of the array. In addition to testing a dilution range for your glycan-binding sample, we recommend setting up control assays for any additional detection or secondary antibodies to ensure that any binding observed is specific to your sample. A fluorescent signal due to specific binding to your sample of interest should be dose-dependent within the dynamic range of your sample dilution, and should have a positive binding signal after a signal from control assays has been subtracted. Calculate the volume of sample needed depending on how many slides and subarrays are to be assayed. Use 80 μL volume of sample per well for 16 subarray cassettes and 150 μL for 8 subarray cassettes to ensure full and even coverage of the printed area throughout incubation. 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 subarrays. 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, and there are no dry spots on the subarray after the sample is added.

Assay Protocol

Part 1 – Blocking

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

1. Allow the arrayed slides to equilibrate to room temperature (20-30 minutes) before opening the moisture barrier bag.
2. Add blocking buffer to each subarray well. We recommend using 100 μL 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.
3. Cover the wells with adhesive film to prevent evaporation and incubate slide on shaker at 85 rpm for 1 hour. 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.

Part 2 – Binding assay

1. Unless the glycan-binding sample of interest is bacteria or cells, centrifuge samples briefly to avoid adding irrelevant particles to the array.
2. Touch the pipette tip to the corner of the well of the cassette and tip the slide so that the sample pools to that corner to remove the sample. Avoid touching the array surface.

3. Immediately apply the glycan-binding sample of interest to each well. We recommend using 100 µL 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. Avoid leaving air bubbles.
4. Seal the wells with adhesive film to prevent evaporation. If the sample is fluorescently labelled, cover with aluminum foil to keep it in the dark. Incubate on the shaker for 1-3 hours at 100 rpm. If the samples can easily aggregate, shake at higher speed to prevent aggregation. Longer incubation time may increase 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 samples are fluorescently labelled, go directly to Part 6 – Final wash and dry.

Part 3 – Wash

1. Touch the pipette tip to the corner of the well of the cassette and tip the slide so that the sample pools to that corner to remove the sample. Avoid touching the array surface, but a gentle touch is okay to ensure no sample is left pooled in the corners.
2. Add wash buffer to each well. We recommend using 100 µL per well for 16 subarray cassettes and 200 µL for 8 subarray cassettes. Cover the wells with adhesive film and incubate on the shaker for 5 minutes at 85 rpm. Completely remove the wash buffer by pipette and repeat this step. Avoid allowing the slide to dry out and have your next wash or sample ready before you remove the wash 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 completely removing the wash buffer immediately add the biotinylated antibody to each well. We recommend using 100 µL per well for 16 subarray cassettes and 200 µL for 8 subarray cassettes. Seal the wells with adhesive film and incubate on the shaker for 1 hour at 100 rpm. 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 wash buffer immediately add the fluorescently labelled streptavidin sample. 100 µL per well is recommended for 16 subarray cassettes and 200 µL for 8 subarray cassettes. Seal the wells with adhesive film and shield the wells from light with aluminum foil. Incubate on the shaker at 85 rpm for 1 hour. Longer incubation time is acceptable, but not necessary.

Part 6 – Final wash and dry

1. Touch the pipette tip to the corner of the well of the cassette and tip the slide so that the sample pools to that corner to remove the sample. Avoid touching the array surface.
2. Briefly rinse each well with wash buffer. 100 µL per well is recommended for 16 subarray cassettes and 200 µL for 8 subarray cassettes.

3. Completely remove the wash 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 wash buffer. 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 the 60 rpm shaker for 10 minutes.
7. Decant the wash 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 60 rpm shaker for 2 minutes.
9. Decant the water from the jar or beaker.
10. Allow the slide to dry completely in a clean, dust free environment before scanning.

Analysis

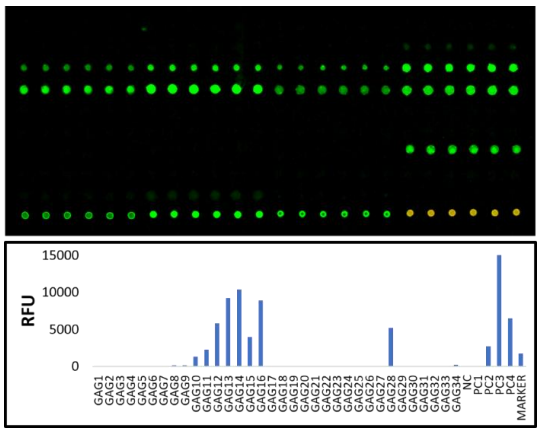
Scan the slide in a laser fluorescence scanner at the wavelength of emission for the fluorophore used. Adjust the laser power and PMT to obtain the highest possible signals without any being saturated (saturated positive control signal is okay). Analyze data with microarray analysis software. If there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

Interpretation of 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. Subtracting the negative control's signal from the other binding signals will give more accurate specific binding data.

Positive Control (a biotinylated probe): This positive control will bind directly to the fluorescent labelled streptavidin. If your glycan-binding sample is already fluorescently labelled, or in any case where the addition of fluorescent labelled streptavidin to the array was not preformed (Part 5 – Fluorescent staining) this positive control will not be reactive.

IgG (PC2, PC3, PC4): IgG is an antibody found in blood that is a primary component of humoral immunity. If the glycan-binding or secondary antibody sample is an anti-IgG from human, rabbit, or mouse it should bind to the respective IgG control. Cross-reactivity of IgGs between species is common.



Marker: The array marker should show fluorescence signal regardless of the assay. It is there primarily to aid with orientation of the array map during analysis.

Typical Binding Assay Result from the Glycosaminoglycan Microarray

Example 1: Glycosaminoglycan Microarray on 8 subarray format. A subarray assayed with antithrombin-III (10 µg/ml), followed by anti-antithrombin-III antibody (2 µg/ml), and finally anti-mouse IgG-

AlexaFluoro555 (10 μg/ml) (sandwich assay format). The array was scanned with InnoScan 710 microarray scanner (XDR mode) at 1 PMT and 100% laser power at 532nm wavelength. The positive control 3 (mouse IgG) shows binding as expected.

Troubleshooting

Condition	Possible Causes	Potential Solutions
High Background	• Concentration of sample of interest 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. Use control assays to determine which sample is causing high background. • 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. Make sure buffers are filtered. • 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