Glycosaminoglycan Microarray User Manual

Z BIOTECH

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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.

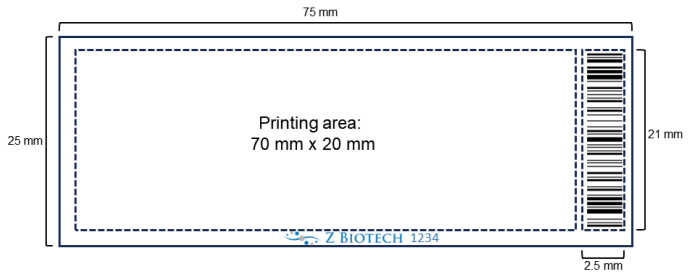
Handling and Storage

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.

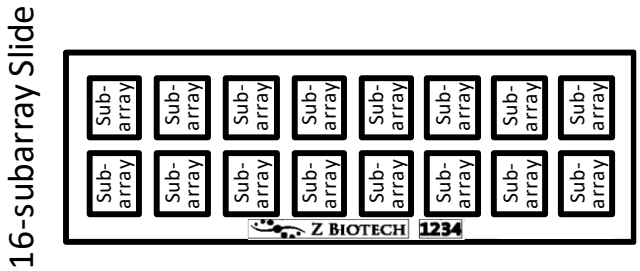
Allow the bag of slides to equilibrate to room temperature at least 20 minutes before opening. After opening, re-seal any unused slides in the moisture barrier bag with a desiccant inside and refreeze.

Array Map/Schematic

Glycosaminoglycan Microarray slides have either 8 or 16 subarrays. Arrays are printed on the side with the "Z Biotech" label 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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GAG13	GAG13	GAG13	GAG13	GAG14	GAG14	GAG14	GAG14	GAG15	GAG15	GAG15	GAG15	GAG16	GAG16	GAG16	GAG16
GAG17	GAG17	GAG17	GAG17	GAG18	GAG18	GAG18	GAG18	GAG19	GAG19	GAG19	GAG19	GAG20	GAG20	GAG20	GAG20
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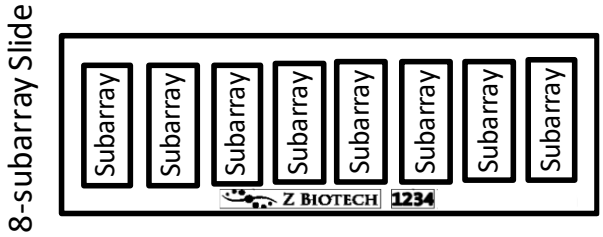
GAG37	GAG37	GAG37	GAG37	GAG38	GAG38	GAG38	GAG38	GAG39	GAG39	GAG39	GAG39	GAG40	GAG40	GAG40	GAG40
GAG41	GAG41	GAG41	GAG41	GAG42	GAG42	GAG42	GAG42	GAG43	GAG43	GAG43	GAG43	GAG44	GAG44	GAG44	GAG44
GAG45	GAG45	GAG45	GAG45	GAG46	GAG46	GAG46	GAG46	NC	NC	NC	NC	PC1	PC1	PC1	PC1
PC2	PC2	PC2	PC2	PC3	PC3	PC3	PC3	PC4	PC4	PC4	PC4	Marker	Marker	Marker	Marker

Array Map (8-subarray slides):



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GAG45	GAG45	GAG45	GAG45	GAG45	GAG45	GAG46	GAG46	GAG46	GAG46	GAG46	GAG46	NC	NC	NC	NC	NC	NC	PC1	PC1	PC1	PC1	PC1	PC1
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)	ΔUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_4$, Mw 3,000
GAG9	Heparin dp12 (H12)	ΔUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_5$, Mw 3,550
GAG10	Heparin dp14 (H14)	ΔUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_6$, Mw 4,100
GAG11	Heparin dp16 (H16)	ΔUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_7$, Mw 4,650
GAG12	Heparin dp18 (H18)	ΔUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_8$, Mw 5,200
GAG13	Heparin dp20 (H20)	ΔUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_9$, Mw 5,750
GAG14	Heparin dp22 (H22)	ΔUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_{10}$, Mw 6,300
GAG15	Heparin dp24 (H24)	ΔUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_{11}$, Mw 6,850
GAG16	Heparin dp30 (H30)	ΔUA,2S - GlcNS,6S– [IdoUA,2S – GlcNS,6S]$_{14}$, Mw 9,000
GAG17	Chondroitin Sulphate Oligosaccharide dp10 (CSO10)	ΔUA - [GalNAc,6S or 4S - GlcA]$_4$ - GalNAc,6S or 4S, Mw 2,480
GAG18	Chondroitin Sulphate Oligosaccharide dp12 (CSO12)	ΔUA - [GalNAc,6S or 4S - GlcA]$_5$ - GalNAc,6S or 4S, Mw 2,976
GAG19	Chondroitin Sulphate Oligosaccharide dp14 (CSO14)	ΔUA - [GalNAc,6S or 4S - GlcA]$_6$ - GalNAc,6S or 4S, Mw 3,472
GAG20	Chondroitin Sulphate Oligosaccharide dp16 (CSO16)	ΔUA - [GalNAc,6S or 4S - GlcA]$_7$ - GalNAc,6S or 4S, Mw 3,968
GAG21	Chondroitin Sulphate Oligosaccharide dp18 (CSO18)	ΔUA - [GalNAc,6S or 4S - GlcA]$_8$ - GalNAc,6S or 4S, Mw 4,464
GAG22	Chondroitin Sulphate Oligosaccharide dp20 (CSO20)	ΔUA - [GalNAc,6S or 4S - GlcA]$_9$ - GalNAc,6S or 4S, Mw 4,960
GAG23	Chondroitin Sulphate D Oligosaccharide dp10 (CSDO10)	ΔUA - [GalNAc,6S or 4S – GlcA +/- 2S]$_4$ – GalNAc,6S, Mw 2,480
GAG24	Chondroitin Sulphate D Oligosaccharide dp12 (CSDO12)	ΔUA - [GalNAc,6S or 4S – GlcA +/- 2S]$_5$ – GalNAc,6S, Mw 2,976
GAG25	Chondroitin Sulphate D Oligosaccharide dp14 (CSDO14)	ΔUA - [GalNAc,6S or 4S – GlcA +/- 2S]$_6$ – GalNAc,6S, Mw 3,472
GAG26	Chondroitin Sulphate D Oligosaccharide dp16 (CSDO16)	ΔUA - [GalNAc,6S or 4S – GlcA +/- 2S]$_7$ – GalNAc,6S, Mw 3,968
GAG27	Chondroitin Sulphate D Oligosaccharide dp18 (CSDO18)	ΔUA - [GalNAc,6S or 4S – GlcA +/- 2S]$_8$ – GalNAc,6S, Mw 4,464
GAG28	Chondroitin Sulphate D Oligosaccharide dp20 (CSDO20)	ΔUA - [GalNAc,6S or 4S – GlcA +/- 2S]$_9$ – GalNAc,6S, Mw 4,960
GAG29	Dermatan Sulphate dp10 (DS10)	ΔUAβ1,3 - GalNAc,4S – (IdoA – GalNAc,4S)$_4$, Mw 2,480
GAG30	Dermatan Sulphate dp12 (DS12)	ΔUAβ1,3 - GalNAc,4S – (IdoA – GalNAc,4S)$_5$, Mw 2,976
GAG31	Dermatan Sulphate dp14 (DS14)	ΔUAβ1,3 - GalNAc,4S – (IdoA – GalNAc,4S)$_6$, Mw 3,472
GAG32	Dermatan Sulphate dp16 (DS16)	ΔUAβ1,3 - GalNAc,4S – (IdoA – GalNAc,4S)$_7$, Mw 3,968
GAG33	Dermatan Sulphate dp18 (DS18)	ΔUAβ1,3 - GalNAc,4S – (IdoA – GalNAc,4S)$_8$, Mw 4,464
GAG34	Dermatan Sulphate dp20 (DS20)	ΔUAβ1,3 - GalNAc,4S – (IdoA – GalNAc,4S)$_9$, Mw 4,960
GAG35	Heparan Sulphate Oligosaccharide dp10 (Hep I, low and intermediate sulphation)	ΔUA - GlcNS/GlcNAc – [UA - GlcNS/GlcNAc]$_4$, Mw 2,800
GAG36	Heparan Sulphate Oligosaccharide dp12 (Hep I, low and intermediate sulphation)	ΔUA - GlcNS/GlcNAc – [UA - GlcNS/GlcNAc]$_5$, Mw 3,500
GAG37	Heparan Sulphate Oligosaccharide dp14 (Hep I, low and intermediate sulphation)	ΔUA - GlcNS/GlcNAc – [UA - GlcNS/GlcNAc]$_6$, Mw 4,000
GAG38	Heparan Sulphate Oligosaccharide dp16 (Hep I, low and intermediate sulphation)	ΔUA - GlcNS/GlcNAc – [UA - GlcNS/GlcNAc]$_7$, Mw 4,400
GAG39	Heparan Sulphate Oligosaccharide dp18 (Hep I, low and intermediate sulphation)	ΔUA - GlcNS/GlcNAc – [UA - GlcNS/GlcNAc]$_8$, Mw 5,000
GAG40	Heparan Sulphate Oligosaccharide dp20 (Hep I, low and intermediate sulphation)	ΔUA - GlcNS/GlcNAc – [UA - GlcNS/GlcNAc]$_9$, Mw 5,400
GAG41	Heparan Sulphate Oligosaccharide dp10 (Hep III, high sulphation)	ΔUA - GlcNS/GlcNAc – [UA - GlcNS/GlcNAc]$_4$, Mw 2,800
GAG42	Heparan Sulphate Oligosaccharide dp12 (Hep III, high sulphation)	ΔUA - GlcNS/GlcNAc – [UA - GlcNS/GlcNAc]$_5$, Mw 3,500
GAG43	Heparan Sulphate Oligosaccharide dp14 (Hep III, high sulphation)	ΔUA - GlcNS/GlcNAc – [UA - GlcNS/GlcNAc]$_6$, Mw 4,200
GAG44	Heparan Sulphate Oligosaccharide dp16 (Hep III, high sulphation)	ΔUA - GlcNS/GlcNAc – [UA - GlcNS/GlcNAc]$_7$, Mw 4,800
GAG45	Heparan Sulphate Oligosaccharide dp18 (Hep III, high sulphation)	ΔUA - GlcNS/GlcNAc – [UA - GlcNS/GlcNAc]$_8$, Mw 5,500
GAG46	Heparan Sulphate Oligosaccharide dp20 (Hep III, high sulphation)	ΔUA - GlcNS/GlcNAc – [UA - GlcNS/GlcNAc]$_9$, Mw 6,200

Hep I: Heparinase I; Hep III: Heparinase III

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 slides
- 16 or 8 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 labelled 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 volume of 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

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 blocking buffer to each subarray well.
3. Cover the wells with adhesive film to prevent evaporation and incubate slide on 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 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 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 labelled, cover 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 at higher speed to prevent protein 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 protein samples are fluorescently labelled, 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 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 completely 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 labelled 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 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 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 glycan binding is saturated) 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 spots on a negative control assay (assay with only detection antibodies and fluorophore) will give more accurate specific binding data.

Interpretation of Control Signals:

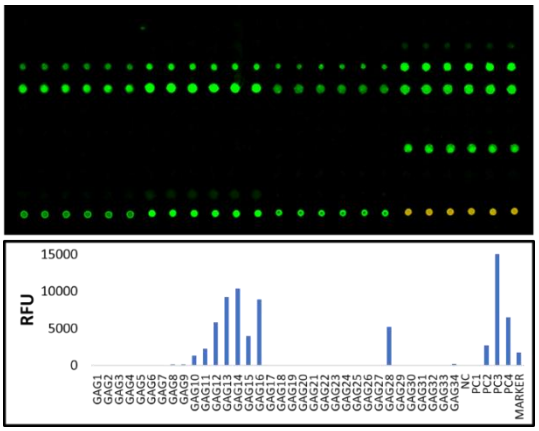
Negative Control (Print Buffer): The negative control should produce a signal close to the intensity of the background. Since there is no binding involved with the negative control, any other signals around the negative control's intensity are also not binding.

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.

Biotinylated Mannose (PC1): This positive control will bind directly to the fluorescent labelled streptavidin. If your glycan-binding protein sample is already fluorescently labelled, or in any case where the addition of fluorescently labelled 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 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.

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