Blood Group Antigen
Glycan Array User Manual

Z BIOTECH

Innovative Biochemical Analysis Solutions

Website: http://www.zbiotech.com/home.html

Tel: (720) 859-3551

Email: info@zbiotech.com

For Research Use Only

Table of Contents

Introduction

Blood group antigens are the glycan structures presented on blood cells and various other proteins, tissues, and secretions that determine blood type, and also indicate the existence certain circulating antibodies that attack foreign blood antigens. The most common blood types are categorized into ‘A’, ‘B’, or ‘O’ depending on the basic structure of the blood antigens, although smaller variations in these structures classify them further into distinct sub-types.

These antigens and blood group antibodies not only help identify blood type cross-reactivity for transfusions and transplants, but they’re also involved in the mediation of pathogens, viruses, and diseased cells. There is correlation between blood type and susceptibility to certain diseases, which indicates that the properties of these antigens and respective circulating antibodies affect the specific and non-specific interactions involved with disease pathogenesis. Research on the underlying dynamics that describe the relationship between disease risk and blood group antigens is currently limited.

The blood group antigen (BGA) glycan array is an advanced, high-throughput tool that allows screening of relatively small amounts of serum samples or other BGA-binding samples of interest to determine the reactivity of antibodies or proteins that would interact with specific blood groups. This tool can aid in providing a more detailed analysis of blood type and donor compatibility, or help researchers investigate the role of blood antigen interactions related to disease.

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.

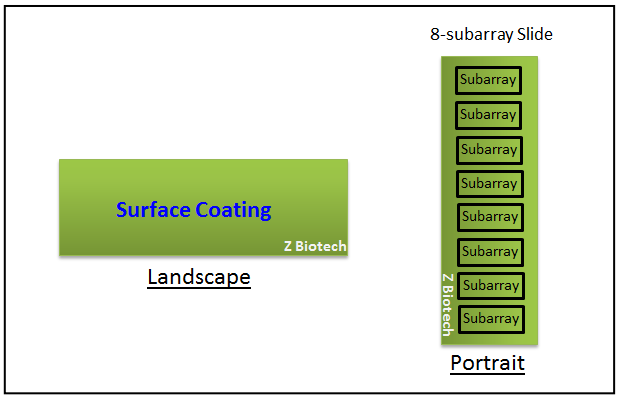
Handling and Storage

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.

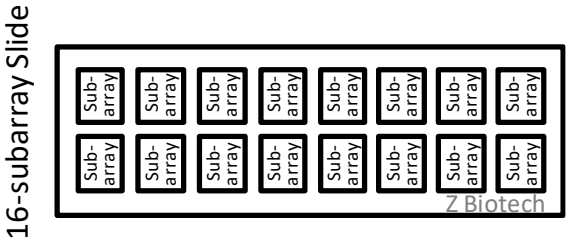
Allow the bag of slides to equilibrate to room temperature at least 20 minutes before opening. After opening, re-seal any unused slides in the moisture barrier bag with a desiccant inside and refreeze. Handle the slides in a clean, dust free environment. Wear gloves and hold the slides on the edges.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

The BGA Glycan Array slides have 8 or 16 subarrays depending on the customer's request. 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 on the following pages.



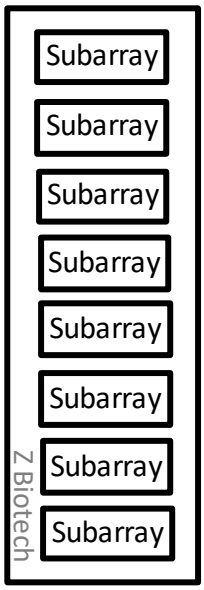
Array Map (16-subarray):



	1	2	3	4	5	6	7	8	9	10	11	12
1	A-1	A-1	A-1	A-2	A-2	A-2	A-3	A-3	A-3	A-4	A-4	A-4
2	A-5	A-5	A-5	A-6	A-6	A-6	A-TRI	A-TRI	A-TRI	B-1	B-1	B-1
3	B-2	B-2	B-2	B-3	B-3	B-3	B-4	B-4	B-4	B-5	B-5	B-5
4	B-6	B-6	B-6	B-TRI	B-TRI	B-TRI	H-1	H-1	H-1	H-2	H-2	H-2
5	H-3	H-3	H-3	H-4	H-4	H-4	H-5	H-5	H-5	H-6	H-6	H-6
6	H-DI	H-DI	H-DI	NC	NC	NC	PC1	PC1	PC1	PC2	PC2	PC2
7	PC3	PC3	PC3	PC4	PC4	PC4	PC5	PC5	PC5	MARK	MARK	MARK

Array Map (8-subarray):

8-subarray Slide

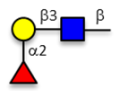
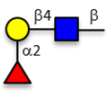
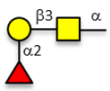
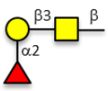
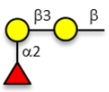
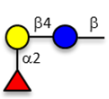
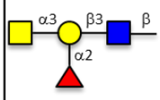
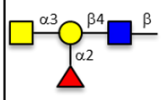
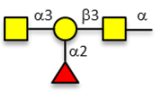
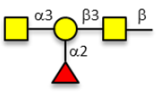
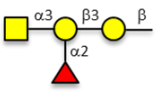
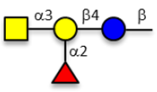
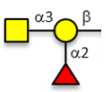
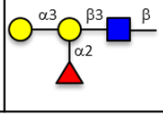
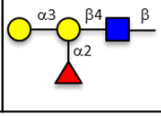
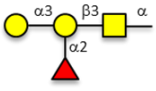
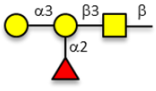
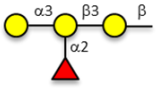
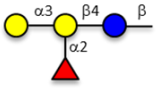
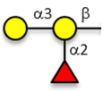


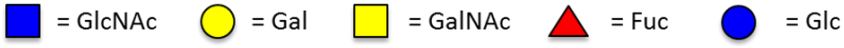
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	A-1	A-1	A-1	A-1	A-1	A-1	A-2	A-2	A-2	A-2	A-2	A-2	A-3	A-3	A-3	A-3	A-3	A-3	A-4	A-4	A-4	A-4	A-4	A-4
2	A-5	A-5	A-5	A-5	A-5	A-5	A-6	A-6	A-6	A-6	A-6	A-6	A-TRI	A-TRI	A-TRI	A-TRI	A-TRI	A-TRI	B-1	B-1	B-1	B-1	B-1	B-1
3	B-2	B-2	B-2	B-2	B-2	B-2	B-3	B-3	B-3	B-3	B-3	B-3	B-4	B-4	B-4	B-4	B-4	B-4	B-5	B-5	B-5	B-5	B-5	B-5
4	B-6	B-6	B-6	B-6	B-6	B-6	B-TRI	B-TRI	B-TRI	B-TRI	B-TRI	B-TRI	H-1	H-1	H-1	H-1	H-1	H-1	H-2	H-2	H-2	H-2	H-2	H-2
5	H-3	H-3	H-3	H-3	H-3	H-3	H-4	H-4	H-4	H-4	H-4	H-4	H-5	H-5	H-5	H-5	H-5	H-5	H-6	H-6	H-6	H-6	H-6	H-6
6	H-DI	H-DI	H-DI	H-DI	H-DI	H-DI	NC	NC	NC	NC	NC	NC	PC1	PC1	PC1	PC1	PC1	PC1	PC2	PC2	PC2	PC2	PC2	PC2
7	PC3	PC3	PC3	PC3	PC3	PC3	PC4	PC4	PC4	PC4	PC4	PC4	PC5	PC5	PC5	PC5	PC5	PC5	MARK	MARK	MARK	MARK	MARK	MARK

Blood Group Antigen Identification List (BSA conjugates):

ID	Structure
A-1	GalNAcα1-3(Fucα1-2)Galβ1-3GlcNAcβ
A-2	GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ
A-3	GalNAcα1-3(Fucα1-2)Galβ1-3GalNAcα
A-4	GalNAcα1-3(Fucα1-2)Galβ1-3GalNAcβ
A-5	GalNAcα1-3(Fucα1-2)Galβ1-3Galβ
A-6	GalNAcα1-3(Fucα1-2)Galβ1-4Glcβ
A-Tri	GalNAcα1-3(Fucα1-2)Galβ
B-1	Galα1-3(Fucα1-2)Galβ1-3GlcNAcβ
B-2	Galα1-3(Fucα1-2)Galβ1-4GlcNAcβ
B-3	Galα1-3(Fucα1-2)Galβ1-3GalNAcα
B-4	Galα1-3(Fucα1-2)Galβ1-3GalNAcβ
B-5	Galα1-3(Fucα1-2)Galβ1-3Galβ
B-6	Galα1-3(Fucα1-2)Galβ1-4Glcβ
B-Tri	Galα1-3(Fucα1-2)Galβ
H-1	Fucα1-2Galβ1-3GlcNAcβ
H-2	Fucα1-2Galβ1-4GlcNAcβ
H-3	Fucα1-2Galβ1-3GalNAcα
H-4	Fucα1-2Galβ1-3GalNAcβ
H-5	Fucα1-2Galβ1-3Galβ
H-6	Fucα1-2Galβ1-4Glcβ
H-Di	Fucα1-2Galβ

Blood Group Antigen Graphical Structures (BSA conjugates):

Group	Type-I	Type-II	Type-III	Type-IV	Type-V	Type-VI	Di/Tri-saccharide
H							
A							
B							



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, Human IgM (0.1 mg/ml)

PC4: Postitive control 4, Human IgA (0.1 mg/ml)

PC5: Postitive control 5, Mouse IgG (0.1 mg/ml)

Array Marker (MARK): 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 cassette
- Blocking Buffer (NGBB Item # 10106)
- Glycan Array Assay Buffer 2 (GAAB2 Item #10111)

Materials above are available for purchase in our BGA Glycan Array Kit, or buffers sold individually
(https://squareup.com/market/glycan-array)

- Wash Buffer: 20 mM Tris-HCl, 150 mM NaCl, 0.05% Tween 20, pH 7.6
- BGA glycan-binding samples of interest
- Secondary antibodies (for sandwich assay format only)
- Fluorescent-labeled streptavidin, or fluorescent-labeled detection antibodies (depending on the sample detection method. Consider the excitation wavelength of your scanner)
- Sterile de-ionized water
- Orbital shaker
- Laser fluorescence scanner (able to scan at the wavelength of your fluorophore)
- Centrifuge
- Pipette and sterile pipette tips
- Sterile centrifuge tubes
- Coplin jar or 250 mL beaker
- Adhesive slide cover film
- Aluminum foil

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

Prepare glycan-binding samples or detection antibodies in a centrifuge tube by diluting with the GAAB2 buffer. For the fluorescently labelled streptavidin we recommend a concentration of 1

μg/mL. For detection antibodies, we recommend a concentration of around 1-10 μg/ml. We generally recommend a range of 100 μg/ml to 0.1 μg/ml concentration for glycan-binding protein samples, or a 1:50 dilution of serum 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. In addition to testing a dilution range for your glycan-binding sample of interest, we recommend setting up control assays for any additional detection or secondary antibodies to ensure that any binding observed is specific to your sample of interest. 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 positive binding signal after signal from control assays has been subtracted. Calculate the volume of sample needed depending on how many slides and subarrays are to be assayed. We recommend using 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. 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, 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 120 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. Remove blocking buffer from each well by gently touching the pipette tip to the corner of the well of the cassette and tip the slide so that the sample pools to that corner. Avoid touching the array surface.
2. Immediately apply assay to each well to rinse the array of remaining blocking buffer. 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.

3. Unless the glycan-binding sample of interest is bacteria or cells, centrifuge samples briefly to avoid adding irrelevant particles to the array.
4. Remove assay buffer from each well by gently touching the pipette tip to the corner of the well of the cassette and tip the slide so that the sample pools to that corner. Avoid touching the array surface.
5. 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.
6. 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. Remove glycan-binding samples from each well by gently touching the pipette tip to the corner of the well of the cassette and tip the slide so that the sample pools to that corner. 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 100 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 secondary antibody (Sandwich Assay Format)

1. Unless the secondary 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

If your secondary antibody sample is fluorescently labeled, go directly to Part 6 – Final wash and dry

Part 5 – Fluorescent staining

1. Centrifuge the fluorescent-labeled detection antibody, or 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 100 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 the pipette tip to the corner of the well of the cassette and tip the slide so that the sample pools to that corner. 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. Files for analysis which can be loaded into compatible analysis software and include spot ID's and array layouts (.gal files) can be provided upon request. 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.

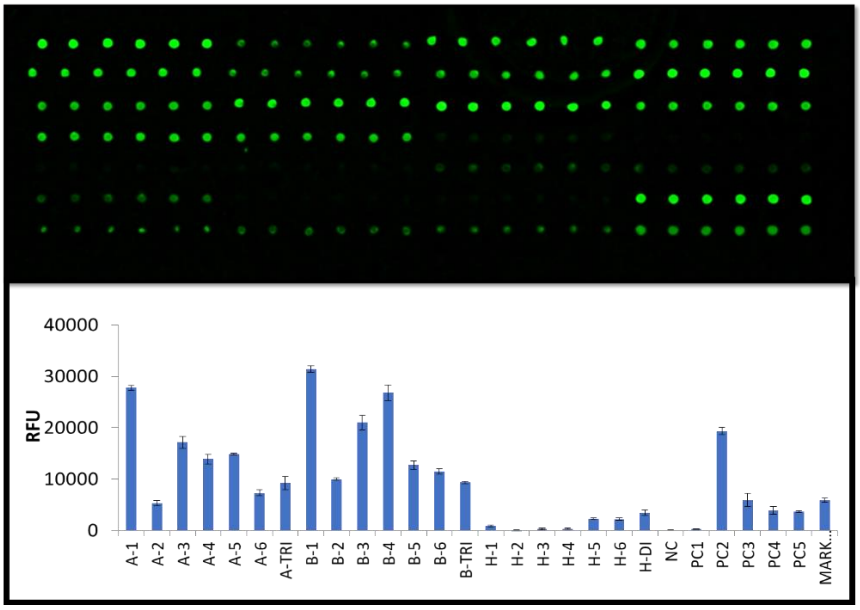
Positive Control (a biotinylated probe): This positive control will bind directly to the fluorescent labelled streptavidin. If your glycan-binding sample is already fluorescently labelled, or in any case where the addition of fluorescent labelled streptavidin to the array was not preformed this positive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

IgG/M/A (PC2, PC3, PC4, PC5): IgG, IgM, or IgA are antibodies found in blood that are primary components of humoral immunity. If the glycan-binding or secondary antibody sample is an anti-IgG, IgM, or IgA from human or mouse it should bind to the respective Ig control. Cross-reactivity of Igs between species is common.

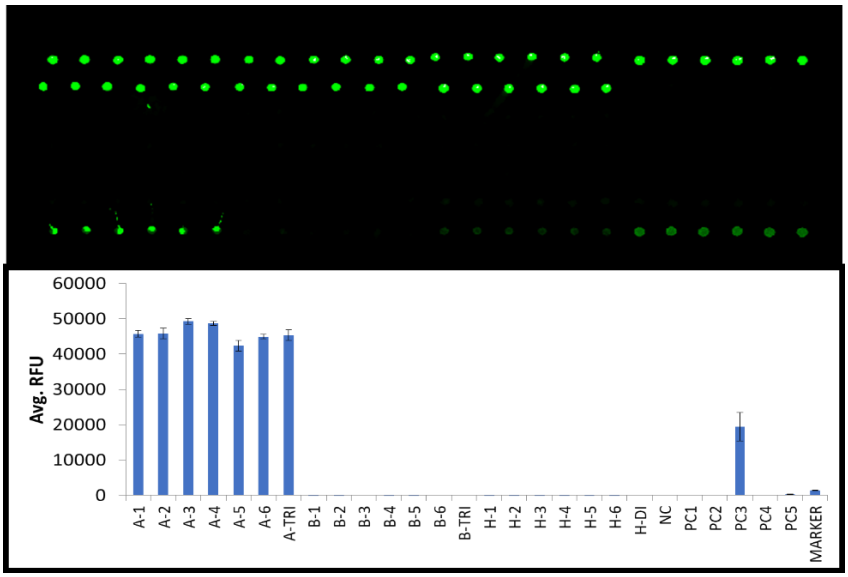
Marker: The array marker should show fluorescence signal regardless of the assay. It is there primarily to aid with orientation of the array map during analysis.

Example Binding Assay Results from the Blood Group Antigen Glycan Microarray

Example 1: A subarray (8-sample format) assayed with human blood serum type O (1:50 dilution), followed by anti-human IgG-Cy3 antibody (5 µg/ml). The array was scanned with InnoScan 710 microarray scanner with low laser power at 1 PMT at 532nm wavelength. The positive control 2 (human IgG) shows binding as well as A and B group antigens. Since the O-type blood group generally is known to have anti-A and anti-B antigen antibodies, binding to A and B group antigens is expected. This data reveals more details of the specific structure subtype reacting with the sera.



Example 2: A subarray (8-sample format) assayed with Anti-A antibody (1:50 dilution), followed by anti-mouse IgM-AF555 antibody (5 µg/ml). The array was scanned with InnoScan 710 microarray scanner with low laser power at 1 PMT at 532nm wavelength. The positive control 3 (human IgM) shows binding as well as all A antigens.



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