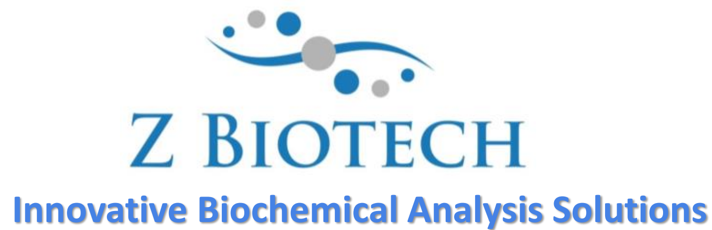
Glycosphingolipid Glycan Array
User Manual



Innovative Biochemical Analysis Solutions

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Introduction

The cells of the nervous system communicate and are identified by the variety of sialylated glycosphingolipids (GSLs) called gangliosides presented on their surface membranes. The species of gangliosides expressed by each cell are incredibly important for brain function and go through dramatic changes during brain development. The expression of specific types of glycosphingolipids correlate with sensory and motor coordination, nerve regeneration, maintaining neuropsychological balance, and many other crucial neurological functions. Aberrant changes such as truncation, over- or under-expression of these gangliosides is involved in neurodegenerative diseases, Tay-Sachs disease, and tumor growth.

These findings emphasize how important the study of these molecules is to the medical research community. Understanding the effects and immunological interactions of aberrantly expressed glycolipids may be key to the development of therapeutic medicines for cancer and neurological disorders. Recently, researchers have begun to recognize the therapeutic potential of administering natural and semisynthetic gangliosides for cancer immunotherapy and neurodegenerative diseases. A large number of new immunotherapies have been using tumor-related gangliosides as promising cancer targets, such as anti-GD2 antibody Dinutuximab for neuroblastoma and anti-Neu5GcGM3 antibody Racotumomab for melanoma. These developments are supported by the emergence of new tools for glycobiology research, including glycan microarrays.

Z Biotech presents a new, sensitive research tool specifically for investigating glycosphingolipids. Our Glycosphingolipid Glycan Array features 58 glycan epitopes representing a variety of glycosphingolipid structures presented on healthy and abnormal cells. On each array slide are 8 or 16 identical subarrays so that as many samples can be analyzed simultaneously, providing sensitive and high-throughput glycan-binding information for antibodies, cells, or other proteins of interest.

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.

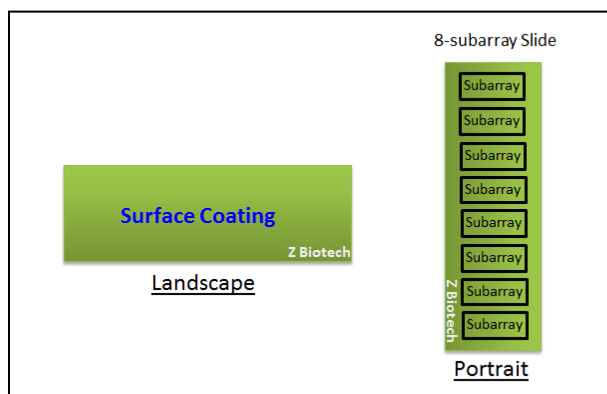
Handling and Storage

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

Glycosphingolipid Glycan Array 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 maps shown:



Array Map (16-subarray slides):

16-subarray Slide
Sub-array Sub-array Sub-array Sub-array Sub-array Sub-array Sub-array Sub-array
Sub-array Sub-array Sub-array Sub-array Sub-array Sub-array Sub-array Sub-array
Z Biotech

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	G1	G1	G1	G1	G2	G2	G2	G2	G3	G3	G3	G3	G4	G4	G4	G4
2	G5	G5	G5	G5	G6	G6	G6	G6	G7	G7	G7	G7	G8	G8	G8	G8
3	G9	G9	G9	G9	G10	G10	G10	G10	G11	G11	G11	G11	G12	G12	G12	G12
4	G13	G13	G13	G13	G14	G14	G14	G14	G15	G15	G15	G15	G16	G16	G16	G16
5	G17	G17	G17	G17	G18	G18	G18	G18	G19	G19	G19	G19	G20	G20	G20	G20
6	G21	G21	G21	G21	G22	G22	G22	G22	G23	G23	G23	G23	G24	G24	G24	G24
7	G25	G25	G25	G25	G26	G26	G26	G26	G27	G27	G27	G27	G28	G28	G28	G28
8	G29	G29	G29	G29	G30	G30	G30	G30	G31	G31	G31	G31	G32	G32	G32	G32
9	G33	G33	G33	G33	G34	G34	G34	G34	G35	G35	G35	G35	G36	G36	G36	G36
10	G37	G37	G37	G37	G38	G38	G38	G38	G39	G39	G39	G39	G40	G40	G40	G40
11	G41	G41	G41	G41	G42	G42	G42	G42	G43	G43	G43	G43	G44	G44	G44	G44
12	G45	G45	G45	G45	G46	G46	G46	G46	G47	G47	G47	G47	G48	G48	G48	G48
13	G49	G49	G49	G49	G50	G50	G50	G50	G51	G51	G51	G51	G52	G52	G52	G52
14	G53	G53	G53	G53	G54	G54	G54	G54	G55	G55	G55	G55	G56	G56	G56	G56
15	G57	G57	G57	G57	G58	G58	G58	G58	NC	NC	NC	NC	PC1	PC1	PC1	PC1
16	PC2	PC2	PC2	PC2	PC3	PC3	PC3	PC3	PC4	PC4	PC4	PC4	MARKER	MARKER	MARKER	MARKER

Array Map (8-subarray slides):

8-subarray Slide
Subarray Subarray Subarray Subarray Subarray Subarray Subarray Subarray
Z Biotech

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	G1	G1	G1	G1	G1	G1	G2	G2	G2	G2	G2	G2	G3	G3	G3	G3	G3	G3	G4	G4	G4	G4	G4	G4	G5	G5	G5	G5	G5	G5
2	G6	G6	G6	G6	G6	G6	G7	G7	G7	G7	G7	G7	G8	G8	G8	G8	G8	G8	G9	G9	G9	G9	G9	G9	G10	G10	G10	G10	G10	G10
3	G11	G11	G11	G11	G11	G11	G12	G12	G12	G12	G12	G12	G13	G13	G13	G13	G13	G13	G14	G14	G14	G14	G14	G14	G15	G15	G15	G15	G15	G15
4	G16	G16	G16	G16	G16	G16	G17	G17	G17	G17	G17	G17	G18	G18	G18	G18	G18	G18	G19	G19	G19	G19	G19	G19	G20	G20	G20	G20	G20	G20
5	G21	G21	G21	G21	G21	G21	G22	G22	G22	G22	G22	G22	G23	G23	G23	G23	G23	G23	G24	G24	G24	G24	G24	G24	G25	G25	G25	G25	G25	G25
6	G26	G26	G26	G26	G26	G26	G27	G27	G27	G27	G27	G27	G28	G28	G28	G28	G28	G28	G29	G29	G29	G29	G29	G29	G30	G30	G30	G30	G30	G30
7	G31	G31	G31	G31	G31	G31	G32	G32	G32	G32	G32	G32	G33	G33	G33	G33	G33	G33	G34	G34	G34	G34	G34	G34	G35	G35	G35	G35	G35	G35
8	G36	G36	G36	G36	G36	G36	G37	G37	G37	G37	G37	G37	G38	G38	G38	G38	G38	G38	G39	G39	G39	G39	G39	G39	G40	G40	G40	G40	G40	G40
9	G41	G41	G41	G41	G41	G41	G42	G42	G42	G42	G42	G42	G43	G43	G43	G43	G43	G43	G44	G44	G44	G44	G44	G44	G45	G45	G45	G45	G45	G45
10	G46	G46	G46	G46	G46	G46	G47	G47	G47	G47	G47	G47	G48	G48	G48	G48	G48	G48	G49	G49	G49	G49	G49	G49	G50	G50	G50	G50	G50	G50
11	G51	G51	G51	G51	G51	G51	G52	G52	G52	G52	G52	G52	G53	G53	G53	G53	G53	G53	G54	G54	G54	G54	G54	G54	G55	G55	G55	G55	G55	G55
12	G56	G56	G56	G56	G56	G56	G57	G57	G57	G57	G57	G57	G58	G58	G58	G58	G58	G58							NC	NC	NC	NC	NC	NC
13	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

Glycosphingolipid Glycan Identification List:

Type	ID	Glycan Structure	Abbrevation Name
Ganglioside	G1	Neu5Acα2–3Galβ1–4Glc	Ac-GM3
	G2	Neu5Gcα2–3Galβ1–4Glc	Gc-GM3
	G3	Kdnα2–3Galβ1–4Glc	Kdn-GM3
	G4	Neu5Ac8Meα2–3Galβ1–4Glc	Ac8Me-GM3
	G5	Neu5Acα2–3(GalNAcβ1–4)Galβ1–4Glc	Ac-GM2
	G6	Neu5Gcα2–3(GalNAcβ1–4)Galβ1–4Glc	Gc-GM2
	G7	Kdnα2–3(GalNAcβ1–4)Galβ1–4Glc	Kdn-GM2
	G8	Neu5Acα2–3(Galβ1–3GalNAcβ1–4)Galβ1–4Glc	Ac-GM1
	G9	Neu5Gcα2–3(Galβ1–3GalNAcβ1–4)Galβ1–4Glc	Gc-GM1
	G10	Kdnα2–3(Galβ1–3GalNAcβ1–4)Galβ1–4Glc	Kdn-GM1
	G11	Neu5Acα2–8Neu5Acα2–3Galβ1–4Glc	Ac-Ac-GD3
	G12	Neu5Acα2–8Neu5Gcα2–3Galβ1–4Glc	Ac-Gc-GD3
	G13	Neu5Acα2–8Kdncα2–3Galβ1–4Glc	Ac-Kdn-GD3
	G14	Neu5Gcα2–8Neu5Acα2–3Galβ1–4Glc	Gc-Ac-GD3
	G15	Neu5Gcα2–8Neu5Gcα2–3Galβ1–4Glc	Gc-Gc-GD3
	G16	Kdnα2–8Neu5Acα2–3Galβ1–4Glc	Kdn-Ac-GD3
	G17	Kdnα2–8Neu5Gcα2–3Galβ1–4Glc	Kdn-Gc-GD3
	G18	Kdnα2–8Kdnα2–3Galβ1–4Glc	Kdn-Kdn-GD3
	G19	Neu5Acα2–8Neu5Acα2–3(GalNAcβ1–4)Galβ1–4Glc	Ac-Ac-GD2
	G20	Neu5Acα2–8Neu5Gcα2–3(GalNAcβ1–4)Galβ1–4Glc	Ac-Gc-GD2
	G21	Neu5Gcα2–8Neu5Acα2–3(GalNAcβ1–4)Galβ1–4Glc	Gc-Ac-GD2
	G22	Neu5Gcα2–8Neu5Gcα2–3(GalNAcβ1–4)Galβ1–4Glc	Gc-Gc-GD2
	G23	Kdnα2–8Neu5Acα2–3(GalNAcβ1–4)Galβ1–4Glc	Kdn-Ac-GD2
	G24	Kdnα2–8Neu5Gcα2–3(GalNAcβ1–4)Galβ1–4Glc	Kdn-Gc-GD2
	G25	Kdnα2–8Kdnα2–3(GalNAcβ1–4)Galβ1–4Glc	Kdn-Kdn-GD2
	G26	Neu5Acα2–3Galβ1–3GalNAcb1–4(Neu5Aca2–3)Galβ1–4Glc	Ac-Ac-GD1a
	G27	Neu5Acα2–8Neu5Acα2–3(Galβ1–3GalNAcβ1–4)Galβ1–4Glc	Ac-Ac-GD1b
	G28	Neu5Gcα2–8Neu5Gcα2–3(Galβ1–3GalNAcβ1–4)Galβ1–4Glc	Gc-Gc-GD1b
	G29	Kdnα2–8Neu5Gcα2–3(Galβ1–3GalNAcβ1–4)Galβ1–4Glc	Kdn-Gc-GD1b
	G30	Neu5Acα2–8Neu5Acα2–3Galβ1–3GalNAcβ1–4(Neu5Aca2–3)Galβ1–4Glc	Ac-Ac-Ac-GT1a
	G31	GalNAcβ1-4(Neu5Acα2–8Neu5Acα2–8Neu5Acα2–3)Galβ1–4Glc	Ac-Ac-Ac-GT2
	G32	Neu5Acα2–8Neu5Acα2–8Neu5Acα2–3Galβ1–4Glc	Ac-Ac-Ac-GT3
Lacto- and Neolacto-series	G33	GlcNAcβ1–3Galβ1–4Glc	Lc_3
	G34	Galβ1–3GlcNAcβ1–3Galβ1–4Glc	Lc_4 (LNT)
	G35	Galβ1–4GlcNAcβ1–3Galβ1–4Glc	nLc_4 (LNnT)
	G36	Galβ1–4(Fucα1–3)GlcNAcβ1–3Galβ1–4Glc	Fuc-nLc_4
	G37	Neu5Acα2–3Galβ1–4GlcNAcβ1–3Galβ1–4Glc	Ac-nLc_4
	G38	Neu5Gcα2–3Galβ1–4GlcNAcβ1–3Galβ1–4Glc	Gc-nLc_4
	G39	Kdnα2–3Galβ1–4GlcNAcβ1–3Galβ1–4Glc	Kdn-nLc_4
	G40	Neu5Ac8Meα2–3Galβ1–4GlcNAcβ1–3Galβ1–4Glc	8MeAc-nLc_4
	G41	Neu5Acα2–3Galβ1–3GlcNAcβ1–3Galβ1–4Glc	Ac-Lc_4
	G42	Neu5Gcα2–3Galβ1–3GlcNAcβ1–3Galβ1–4Glc	Gc-Lc_4
	G43	Kdnα2–3Galβ1–3GlcNAcβ1–3Galβ1–4Glc	Kdn-Lc_4
	G44	Neu5Ac8Meα2–3Galβ1–3GlcNAcβ1–3Galβ1–4Glc	8MeAc-Lc_4
	G45	Neu5Gcα2–3Galβ1–4(Fucα1–3)GlcNAcβ1–3Galβ1–4Glc	Gc-Fuc-nLc_4
	G46	Kdnα2–3Galβ1–4(Fucα1–3)GlcNAcβ1–3Galβ1–4Glc	Kdn-Fuc-nLc_4
Globo- and Isoglobo-series	G47	Galα1–4Galβ1–4Glc	Gb_3
	G48	Galα1–3Galβ1–4Glc	iGb_3
	G49	GalNAcβ1–3Galα1–4Galβ1–4Glc	Gb_4
	G50	GalNAcβ1–3Galα1–3Galβ1–4Glc	iGb_4
	G51	Galβ1–3GalNAcβ1–3Galα1–4Galβ1–4Glc	Gb_5 (SSEA-3)
	G52	Galβ1–3GalNAcβ1–3Galα1–3Galβ1–4Glc	iGb_5
	G53	Fucα1-2Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glc	Globo-H
	G54	Neu5Gcα2–3Galβ1–3GalNAcβ1–3Galα1–4Galβ1–4Glc	Gc-Gb_5
	G55	Kdnα2–3Galβ1–3GalNAcβ1–3Galα1–4Galβ1–4Glc	Kdn-Gb_5
	G56	Neu5Acα2–3Galβ1–3GalNAcβ1–3Galα1–3Galβ1–4Glc	Ac-iGb_5
	G57	Neu5Gcα2–3Galβ1–3GalNAcβ1–3Galα1–3Galβ1–4Glc	Gc-iGb_5
	G58	Kdnα2–3Galβ1–3GalNAcβ1–3Galα1–3Galβ1–4Glc	Kdn-iGb_5

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, 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 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. 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 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. 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 blocking buffer. 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 it. 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. 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. 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 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.

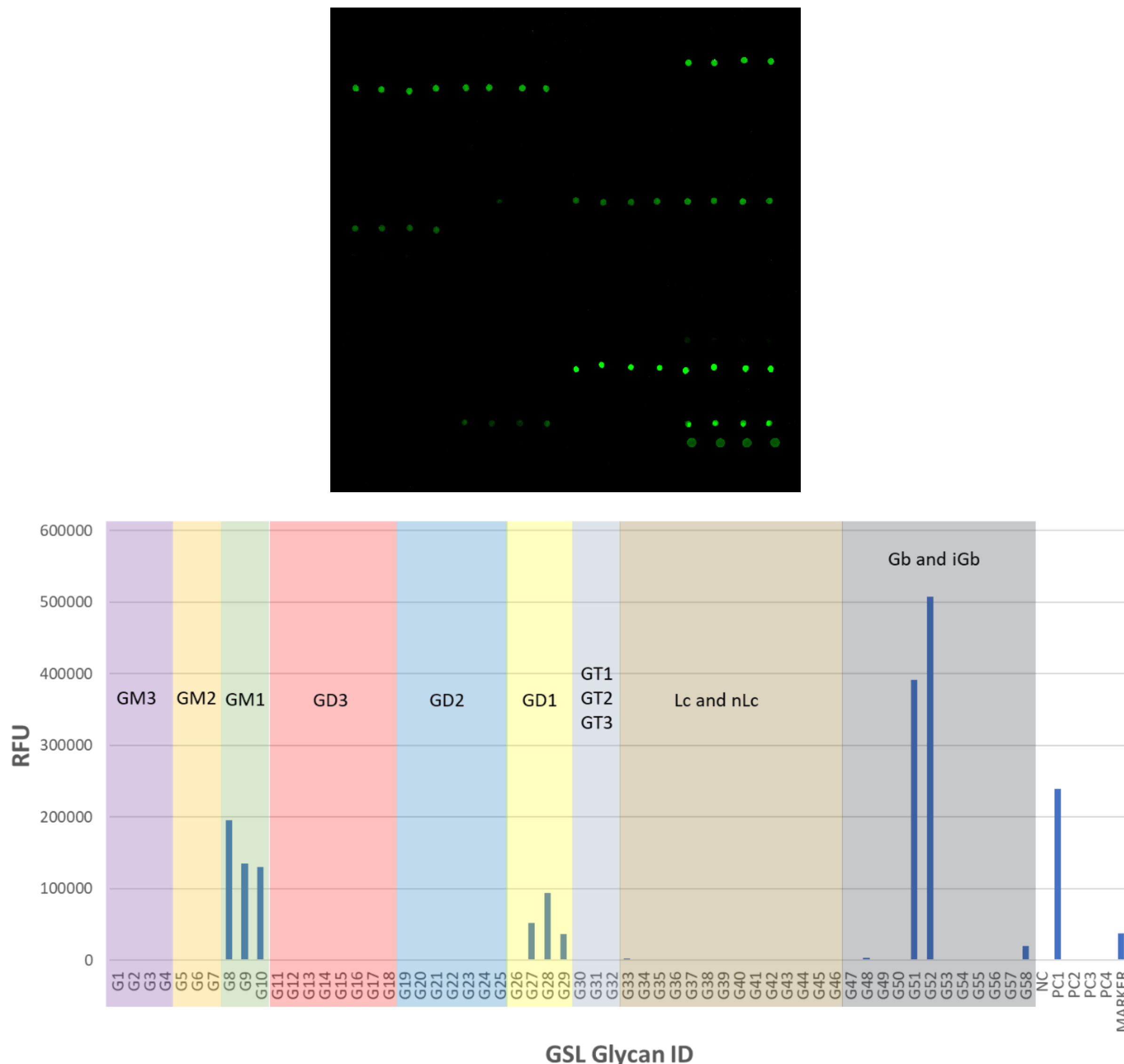
Positive Control (a biotinylated probe): This positive control will bind directly to the fluorescent labelled streptavidin. If your glycan-binding sample is already fluorescently labelled, or in any case where the addition of fluorescent labelled streptavidin to the array was not preformed (Part 5 –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

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 Glycosphingolipid Glycan Microarray

Example 1: Glycosphingolipid Glycan Microarray on 16 subarray formats. A subarray assayed with biotinylated PNA lectin (10 µg/ml), followed by streptavidin-Cy3 (1 µg/ml). The array was scanned with InnoScan 710 microarray scanner (XDR mode) at 1 PMT and 100% laser power at 532nm wavelength. The positive control 1 shows binding as expected.



Troubleshooting

Condition	Possible Causes	Potential Solutions
High Background	• Concentration of 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. 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