100 Glycan Array User Manual

Z BIOTECH

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For Research Use Only

Introduction

Glycans attached to cell membranes and other types of proteins are the primary determinants for binding activity and consecutive cellular function. The study of the function and characteristics of these sugars is a fundamental part of immunology research and is applicable in understanding a variety of intercellular interactions. New technologies such as microarrays for glycan-binding applications allow researchers to investigate and reveal new information about the wide and developing field of glycoscience.

Z Biotech's 100 Glycan Array is used as a general test to help researchers determine binding characteristics of antibodies, proteins, bacteria, cell cultures, or other potential biological samples to an array of 100 glycans representing the range of glycans found glycosylated to cell membranes. Our microarray slides are specially coated to be capable of immobilizing natural glycans with close-ring structure at their free-reducing end.

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.

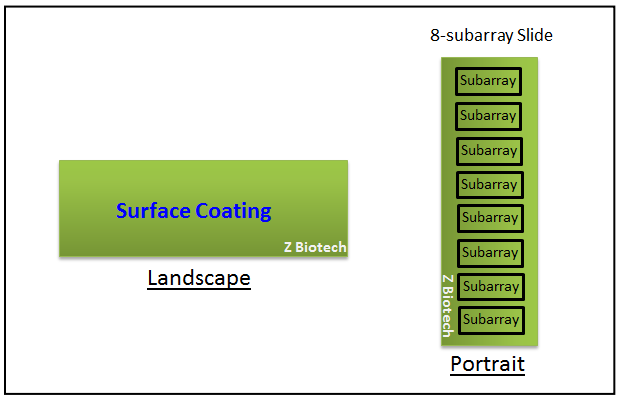
Handling and Storage

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

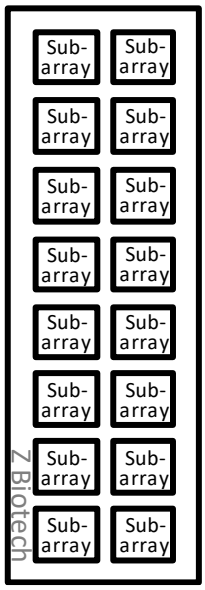
Allow the bag of slides to equilibrate to room temperature at least 20 minutes before opening. After opening, re-seal any unused slides in the moisture barrier bag with a desiccant inside and refreeze.

Array Map/Schematic

100 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 (the "marker" spots will be on the lower right of each array when looking at the slide via the portrait orientation). Dimensions and array maps are shown:



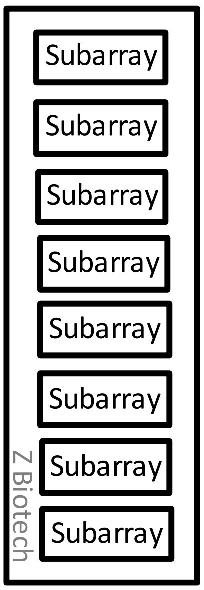
16-subarray Slide



Array Map:

1	1	2	2	3	3	4	4	5	5	6	6	7	7	Marker
8	8	9	9	10	10	11	11	12	12	13	13	14	14	
15	15	16	16	17	17	18	18	19	19	20	20	21	21	
22	22	23	23	24	24	25	25	26	26	27	27	28	28	
29	29	30	30	31	31	32	32	33	33	34	34	35	35	
36	36	37	37	38	38	39	39	40	40	41	41	42	42	
43	43	44	44	45	45	46	46	47	47	48	48	49	49	
50	50	51	51	52	52	53	53	54	54	55	55	56	56	
57	57	58	58	59	59	60	60	61	61	62	62	63	63	
64	64	65	65	66	66	67	67	68	68	69	69	70	70	
71	71	72	72	73	73	74	74	75	75	76	76	77	77	
78	78	79	79	80	80	81	81	82	82	83	83	84	84	
85	85	86	86	87	87	88	88	89	89	90	90	91	91	
92	92	93	93	94	94	95	95	96	96	97	97	98	98	
99	99	100	100	NC	NC	PC1	PC1	PC2	PC2	PC3	PC3	PC4	PC4	Marker

8-subarray Slide



Array Map:

1	1	1	2	2	2	3	3	3	4	4	4	5	5	5	6	6	6	7	7	7	8	8	8	9	9	9
10	10	10	11	11	11	12	12	12	13	13	13	14	14	14	15	15	15	16	16	16	17	17	17	18	18	18
19	19	19	20	20	20	21	21	21	22	22	22	23	23	23	24	24	24	25	25	25	26	26	26	27	27	27
28	28	28	29	29	29	30	30	30	31	31	31	32	32	32	33	33	33	34	34	34	35	35	35	36	36	36
37	37	37	38	38	38	39	39	39	40	40	40	41	41	41	42	42	42	43	43	43	44	44	44	45	45	45
46	46	46	47	47	47	48	48	48	49	49	49	50	50	50	51	51	51	52	52	52	53	53	53	54	54	54
55	55	55	56	56	56	57	57	57	58	58	58	59	59	59	60	60	60	61	61	61	62	62	62	63	63	63
64	64	64	65	65	65	66	66	66	67	67	67	68	68	68	69	69	69	70	70	70	71	71	71	72	72	72
73	73	73	74	74	74	75	75	75	76	76	76	77	77	77	78	78	78	79	79	79	80	80	80	81	81	81
82	82	82	83	83	83	84	84	84	85	85	85	86	86	86	87	87	87	88	88	88	89	89	89	90	90	90
91	91	91	92	92	92	93	93	93	94	94	94	95	95	95	96	96	96	97	97	97	98	98	98	99	99	99
100	100	100	NC	NC	NC	PC1	PC1	PC1	PC2	PC2	PC2	PC3	PC3	PC3	PC4	PC4	PC4								Marker	Marker

100 Glycan Identification List:

No.	Glycan Structure	Name
1	D-Mannose	D-Mannose
2	D-Glucose	D-Glucose
3	D-Fucose	D-Fucose
4	D-Galactose	D-Galactose
5	L-Fucose	L-Fucose
6	L-Rhamnose	L-Rhamnose
7	D-ManNAc	D-ManNAc
8	D-GlcNAc	D-GlcNAc
9	Neu5Ac	Sialic acid
10	Galβ1-4(Fucα1-3)Glc	3FL
11	Galβ1-4Glc	Lactose
12	Galβ1-4GlcNAc	LacNAc
13	Galβ1-3GlcNAc	Lac-N-biose
14	Galβ1-4Gal	4-β-Galactobiose
15	GalNAcβ1-3Gal	β-D-N-acetyl-galactosaminyl 1-3 galactose
16	Glcα1-4Glc	Maltose
17	Glcβ1-4Glc	Cellobiose
18	GlcNAcβ1-4GlcNAc	Diacetylchitobiose
19	GlcNAcβ1-2Man	β-D-N-acetylglucosaminyl 1-2 mannose
20	GlcNAcβ1-4MurNAc	GlcNAcMurNAc
21	GlcNβ1-4GlcN	Chitobiose
22	Manα1-2Man	2-Mannbiose
23	Manα1-3Man	3-Mannobiose
24	Manα1-4Man	4-Mannobiose
25	Manα1-6Man	6-Mannobiose
26	Fucα1-2Gal	Blood H Disaccharide
27	ΔGlcA(2S)α1-4GlcNS(6S)	Heparin Disaccharide
28	GlcNβ1-4GlcNβ1-4GlcN	Chitotriose
29	Galβ1-4Galβ1-4Glc	Globotriose (P^K antigen)
30	GlcNAcβ1-4GlcNAcβ1-4GlcNAc	Triacetyl chitotriose
31	Neu5Acα2-3Galβ1-4Glc	3'-SL (GM3 Glycan)
32	Neu5Acα2-6Galβ1-4Glc	6'-SL
33	Neu5Acα2-3Galβ1-4GlcNAc	3'-SLN
34	Neu5Acα2-6Galβ1-4GlcNAc	6'-SLN
35	(3S)Galβ1-4(Fucα1-3)GlcNAc	Sulpho-Lewis x
36	(3S)Galβ1-4(Fucα1-4)GlcNAc	Sulpho-Lewis a
37	Galβ1-4(Fucα1-3)GlcNAc	Lewis x Trisaccharide
38	Galβ1-3(Fucα1-4)GlcNAc	Lewis a Trisaccharide
39	Glcα1-4Glcα1-4Glc	Maltotriose
40	Glcβ1-4Glcβ1-4Glc	Cellotriose
41	Fucα1-2Galβ1-4Glc	2'FL
42	Galβ1-3Galβ1-3GlcNAc	Linear B-2 Tri (Blood Group B Type 2 Linear Tri)
43	Galα1-4Galβ1-4GlcNAc	P1 antigen Tri
44	Fucα1-2Galβ1-3GlcNAc	Blood group H Trisaccharide
45	GalNAcα1-3-(Fucα1-2)Gal	Blood group A Trisaccharide
46	Galα1-3(Fucα1-2)Gal	Blood group B Trisaccharide
47	Galβ1-3GlcNAcβ1-3Galβ1-4Glc	Lacto-N-tetraose (LNT)
48	Galβ1-4GlcNAcβ1-3Galβ1-4Glc	Lacto-N-neotetraose (LNnT)
49	Galα1-3Galβ1-4Galβ1-3Gal	Gal4
50	Glcα1-4Glcα1-4Glcα1-4Glc	Maltotetratose

51	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAc	Sialyl Lewis x
52	Neu5Acα2-3Galβ1-3(Fucα1-4)GlcNAc	Sialyl Lewis a
53	Fucα1-2Galβ1-3(Fucα1-4)GlcNAc	Lewis b Tetrasaccharide
54	Fucα1-2Galβ1-4(Fucα1-3)GlcNAc	Lewis y Tetrasaccharide
55	Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc	Lacto-N-Fucopentaose I (LNFP-I)
56	Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glc	Lacto-N-Fucopentaose II (LNFP-II)
57	Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc	Lacto-N-Fucopentaose III (LNFP-III)
58	Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-3Gal	Lewis Y Pentasaccharide
59	Glcα1-4Glcα1-4Glcα1-4Glcα1-4Glc	Maltopentaose
60	Manα1-6(Manα1-3)Manα1-6(Manα1-3)Man	Man5
61	Neu5Acα2-6(Galβ1-3)GlcNAcβ1-3Galβ1-4Glc	LS-Tetrasaccharide b (LsTb)
62	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4Glc	LS-Tetrasaccharide c (LsTc)
63	Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glc	GM1 Glycan
64	GalNAcα1-3(Fucα1-2)Galβ1-4(Fucα1-3)Glc	Blood Group A Pentasaccharide
65	GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)Man	Biantennary N-linked Core Pentasaccharide
66	Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4(Fucα1-3)Glc	Lacto-N-difucohexaose II (LNDFH II)
67	Neu5Acα2-3Galβ1-3(Neu5Acα2-6)GlcNAcβ1-3Galβ1-4Glc	DSLNT
68	GlcNAcβ1-4GlcNAcβ1-4GlcNAcβ1-4GlcNAcβ1-4GlcNAcβ1-4GlcNAc	Hexaacetyl Chitohexaose
69	Glcα1-4Glcα1-4Glcα1-4Glcα1-4Glcα1-4Glc	Maltohexaose
70	Glcα1-4Glcα1-4Glcα1-4Glcα1-4Glcα1-4Glcα1-4Glc	Maltoheptaose
71	GlcAβ1-4(6S)GlcNAcα1-4GlcAβ1-4(6S)GlcNAcα1-4GlcAβ1-4(6S)GlcNAcα1-4GlcAβ1-4(6S)GlcNAcα	Heparin Octasaccharide
72	Manα1-6(Manα1-3)Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc	MAN-5; (Man)5(GlcNAc)2
73	Manα1-2Manα1-6(Manα1-2Manα1-3)Manα1-6(Manα1-2Manα1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc	MAN-9; (Man)9(GlcNAc)2
74	GalNAcα1-3(Fucα1-2)Galβ1-3GlcNAc	Blood Group A Type 1 Tetrasaccharide
75	GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAc	Blood Group A Type 2 Tetrasaccharide
76	GalNAcα1-3(Fucα1-2)Galβ1-3GalNAc	Blood Group A Type 3/4 Tetrasaccharide
77	Galα1-3-(Fucα1-2)Galβ1-4(Fucα1-3)Glc	Blood Group B Pentasaccharide
78	Galα1-3-(Fucα1-2)Galβ1-3GlcNAc	Blood Group B Type 1 Tetrasaccharide
79	Galα1-3-(Fucα1-2)Galβ1-4GlcNAc	Blood Group B Type 2 Tetrasaccharide
80	Galα1-3-(Fucα1-2)Galβ1-3GalNAc	Blood Group B Type 3/4 Tetrasaccharide
81	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Gal	Sialyl Lewis x Pentasaccharide
82	Neu5Acα2-3(GalNAcβ1-4)Galβ1-4Glc	GM2 Glycan
83	Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc	LS-Tetrasaccharide a (LsTa)
84	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc	LS-Tetrasaccharide d (LsTd)
85	Neu5Acα2-3Galβ1-3GalNAcβ1-3Gal	Stage-specific Embryonic Antigen 4 (SSEA-4)
86	Glcβ1-4Glcβ1-4Glcβ1-4Glc	Cellotetraose
87	Glcβ1-4Glcβ1-4Glcβ1-4Glcβ1-4Glc	Cellopentaose
88	Glcβ1-4Glcβ1-4Glcβ1-4Glcβ1-4Glcβ1-4Glc	Cellohexaose
89	D-GalNAc	D-GalNAc
90	Galβ1-3GalNAc	T antigen (Core 1)
91	GalNAcα1-3Gal	Adi
92	GalNAcα1-3Galβ1-4Glc	α-D-N-Acetylgalactosaminyl 1-3 galactose β 1-4 glucose
93	Galβ1-6Gal	β 1-6 galactobiose
94	GalNAcα1-3GalNAc	Forssman disaccharide
95	GalNAcβ1-4Gal	Receptor for pili of Pseudomonas aeruginosa
96	Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glc	Stage-specific Embryonic Antigen 3 (SSEA-3)
97	Fucα1-2Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glc	Globo-H
98	Manα1-6(Manα1-3)Manα1-6(Manα1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc	MAN-6; (Man)6(GlcNAc)2
99	[Manα1-2]Manα1-6(Manα1-3)Manα1-6(Manα1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc (3 isomers)	MAN-7; (Man)7(GlcNAc)2
100	[Manα1-2] [Manα1-2] Manα1-6(Manα1-3)Manα1-6(Manα1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc (3 isomers)	MAN-8; (Man)8(GlcNAc)2

Controls

NC: Negative control, Print Buffer

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

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

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

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

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

Materials Required

- Arrayed glass slides
- 16 or 8 cassettes

- Blocking Buffer (Item #10109): 1% BSA in PBST (PBS with 0.05% (v/v) Tween-20, pH 7.4)
- Glycan Array Assay Buffer 2
- Wash Buffer: 20 mM Tris-HCl, 150 mM NaCl, 0.05% Tween 20, pH 7.6
- Laser fluorescence scanner (able to scan at the wavelength of your fluorophore)
- Coplin jar
- Adhesive slide cover film

Preparation of assay samples:

Prepare a centrifuge tube by diluting samples 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 can be accomplished by applying a different dilution of samples to different wells of the array. For the fluorescent 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. Use 100 μL volume of sample per well for 16 subarray cassettes and 200 μL for 8 subarray cassettes in order to ensure full and even coverage of the printed area throughout incubation. If necessary, the assay can still work 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, resulting 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. 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 in order 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 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. 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 liquid. Avoid touching the array surface.
3. Immediately apply the glycan binding protein sample to each well. In order to ensure full and even coverage, 100 μL per well for 16 subarray cassettes and 200 μL for 8 subarrays. 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 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. 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. Avoid touching the array surface.
2. Add wash buffer to each well. We suggest 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 sample is bacteria or cells, centrifuge it briefly to avoid adding irrelevant particles to the array.
2. After completely removing the wash buffer immediately add the secondary 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. Shaking it at 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 the fluorescent labeled streptavidin samples briefly to avoid adding irrelevant particles to the array.
2. After completely removing the wash buffer immediately add the 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.

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 and remove it. Avoid touching the array surface.
2. 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. Analyze data with microarray analysis software. If there is specific binding the signal intensity should be significantly higher than the background signal (area where there are no printed spots). Our standard method of comparing signal intensities is to quantify the median signal intensity data and subtract the background intensity.

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.

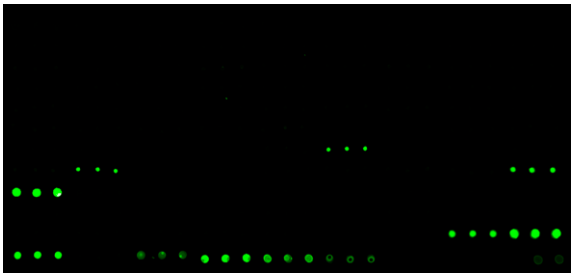
Marker: The array marker should show fluorescent signals regardless of the assay. It aids with orientation of the array map during analysis.

Biotinylated Mannose (PC1): This positive control will bind directly to the fluorescently labelled streptavidin. If your glycan-binding protein sample is already fluorescently labelled, or in any case where the addition of the streptavidin to the array was not preformed (Part 5 – Fluorescent staining) this positive control will not be reactive.

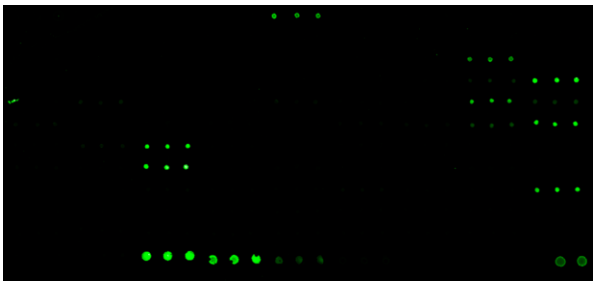
IgG (PC2, PC3, PC4): IgG is an antibody found in blood that is a primary component of humoral immunity. If the glycan-binding or secondary antibody sample is an anti-IgG from human, rabbit, or mouse it should bind to the respective IgG control.

Typical Binding Assay Result from the 100 Glycan Array

Example 1: The 100 glycan array on 8 subarrays format. A subarray assayed with glycan-binding protein biotinylated ConA lectin (10 µg/ml), followed by Streptavidin-Cy3. Array was scanned with GenePix scanner at 450 PMT and 100% laser power at 532nm wavelength. All positive controls 1-4 and the marker show binding as expected, as well as mannose-containing glycans 60, 65, 72, 73, 98, 99, and 100.



Example 2: The 100 glycan array on 8 subarrays format. A subarray assayed with glycan-binding protein biotinylated AAL lectin (1 µg/ml), followed by Streptavidin-Cy3. Array was scanned with GenePix scanner at 500 PMT and 100% laser power at 532nm wavelength. All positive controls 1-4 and the marker show binding as expected, as well as fucose-containing glycans 5, 26, 35, 36, 38, 44, 45, 46, 53, 54, 56, 57, 66 and 81.



Troubleshooting

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