O-Glycan Array User Manual

Z BIOTECH

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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 microarray for glycan-binding applications allow researchers to investigate and reveal new information about this wide and developing field of glycoscience.

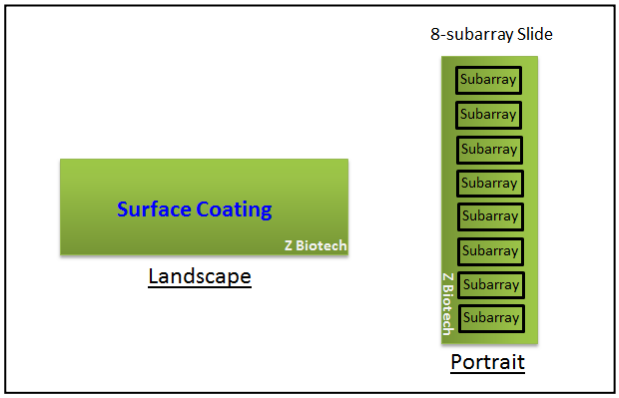
Tumor-associated carbohydrate antigens (TACAs) represent potential biomarkers for early detection of cancer as well as immunotherapeutic targets. For example, the extracellular glycoprotein MUC1 is overexpressed and aberrantly glycosylated in many types of cancers. The extracellular domain of the MUC-1 contains a variable number of tandem repeats (VNTR) of 20 amino acids residues with serine (Ser) or threonine (Thr) sites for O-glycosylation. Generally, aberrantly expressed O-Glycans aid in the metastasis of diseased cells, yet also distinguish diseased from healthy cells at the cell surface. Therefore, the O-Glycan array can serve as a research tool for determining O-glycan immunodominant epitopes in cancers or sensitively detect the glycan binding epitopes of autoantibodies that target unique O-glycan antigens.

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 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.

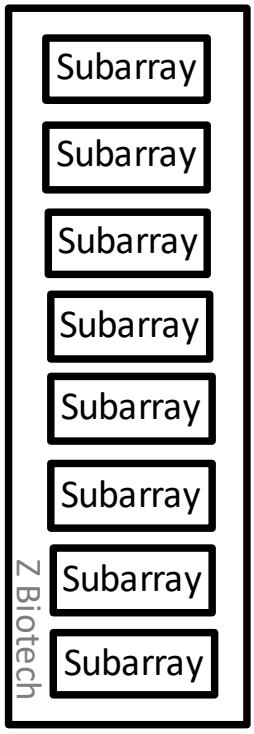


Array Map/Schematic

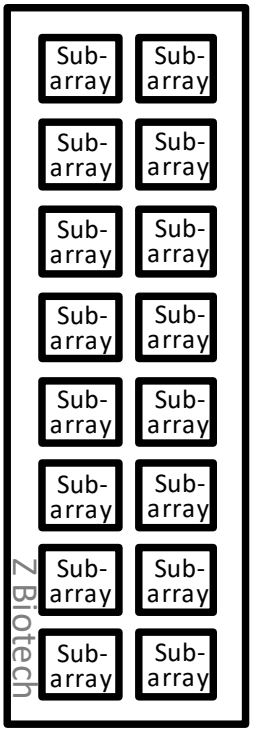
O-Glycan Array slides have 8 or 16 subarrays depending on the customer's request. Arrays are printed on the side with the "Z Biotech" label facing upward. The "Z Biotech" label is located on the bottom right corner from a landscape point of view, or the bottom left corner in a portrait point of view (see image on right). The orientation of the printed array is consistent with

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

8-subarray Slide



16-subarray Slide



Array Map (16-subarray slides)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1	1	1	2	2	2	3	3	3	4	4	4	5	5	5	6	6	6
2	7	7	7	8	8	8	9	9	9	10	10	10	11	11	11	12	12	12
3	13	13	13	14	14	14	15	15	15	16	16	16	17	17	17	18	18	18
4	19	19	19	20	20	20	21	21	21	22	22	22	23	23	23	24	24	24
5	25	25	25	26	26	26	27	27	27	28	28	28	29	29	29	30	30	30
6	31	31	31	32	32	32	33	33	33	34	34	34	35	35	35	36	36	36
7	37	37	37	38	38	38	39	39	39	40	40	40	41	41	41	42	42	42
8	43	43	43	44	44	44	45	45	45	46	46	46	47	47	47	48	48	48
9	49	49	49	50	50	50	51	51	51	52	52	52	53	53	53	54	54	54
10	55	55	55	56	56	56	57	57	57	58	58	58	59	59	59	60	60	60
11	61	61	61	62	62	62	63	63	63	64	64	64	65	65	65	66	66	66
12	67	67	67	68	68	68	69	69	69	70	70	70	71	71	71	72	72	72
13	73	73	73	74	74	74	75	75	75	76	76	76	77	77	77	78	78	78
14	79	79	79	80	80	80	81	81	81	82	82	82	83	83	83	84	84	84
15	85	85	85	86	86	86	87	87	87	88	88	88	89	89	89	90	90	90
16	91	91	91	92	92	92	93	93	93	94	94	94	NC1	NC1	NC1	NC2	NC2	NC2
17	PC1	PC1	PC1	PC2	PC2	PC2	PC3	PC3	PC3	PC4	PC4	PC4				MARKER	MARKER	MARKER

Array Map (8-subarray slides)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	1	1	1	1	2	2	2	2	3	3	3	3	4	4	4	4	5	5	5	5	6	6	6	6	7	7	7	7	92	92
2	8	8	8	8	9	9	9	9	10	10	10	10	11	11	11	11	12	12	12	12	13	13	13	13	14	14	14	14	92	92
3	15	15	15	15	16	16	16	16	17	17	17	17	18	18	18	18	19	19	19	19	20	20	20	20	21	21	21	21	93	93
4	22	22	22	22	23	23	23	23	24	24	24	24	25	25	25	25	26	26	26	26	27	27	27	27	28	28	28	28	93	93
5	29	29	29	29	30	30	30	30	31	31	31	31	32	32	32	32	33	33	33	33	34	34	34	34	35	35	35	35	94	94
6	36	36	36	36	37	37	37	37	38	38	38	38	39	39	39	39	40	40	40	40	41	41	41	41	42	42	42	42	94	94
7	43	43	43	43	44	44	44	44	45	45	45	45	46	46	46	46	47	47	47	47	48	48	48	48	49	49	49	49	NC1	NC1
8	50	50	50	50	51	51	51	51	52	52	52	52	53	53	53	53	54	54	54	54	55	55	55	55	56	56	56	56	NC2	NC2
9	57	57	57	57	58	58	58	58	59	59	59	59	60	60	60	60	61	61	61	61	62	62	62	62	63	63	63	63	PC1	PC1
10	64	64	64	64	65	65	65	65	66	66	66	66	67	67	67	67	68	68	68	68	69	69	69	69	70	70	70	70	PC2	PC2
11	71	71	71	71	72	72	72	72	73	73	73	73	74	74	74	74	75	75	75	75	76	76	76	76	77	77	77	77	PC3	PC3
12	78	78	78	78	79	79	79	79	80	80	80	80	81	81	81	81	82	82	82	82	83	83	83	83	84	84	84	84	PC4	PC4
13	85	85	85	85	86	86	86	86	87	87	87	87	88	88	88	88	89	89	89	89	90	90	90	90	91	91	91	91	MARKER	MARKER

O-Glycan Identification List

O1	GalNAcα-Ser
O2	GalNAcα-Thr
O3	Neu5Acα2-6GalNAcα-Ser
O4	Neu5Acα2-6GalNAcα-Thr
O5	Galβ1-3GalNAcα-Ser
O6	Galβ1-3GalNAcα-Thr
O7	Neu5Acα2-3Galβ1-3GalNAcα-Ser
O8	Neu5Gcα2-3Galβ1-3GalNAcα-Ser
O9	GalNAcβ1-4(Neu5Acα2-3)GalNAcα-Ser
O10	Fucα1-2Galβ1-3GalNAcα-Ser
O11	GalNAcβ1-3(Fucα1-2)Galβ1-3GalNAcα-Ser
O12	Galα1-3(Fucα1-2)Galβ1-3GalNAcα-Ser
O13	GlcNAcβ1-3Galβ1-3GalNAcα-Ser
O14	Galβ1-4GlcNAcβ1-3Galβ1-3GalNAcα-Ser
O15	Galα1-3Galβ1-4GlcNAcβ1-3Galβ1-3GalNAcα-Ser
O16	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-3GalNAcα-Ser
O17	GalNAcβ1-4(Neu5Acα2-3)Galβ1-4GlcNAcβ1-3Galβ1-3GalNAcα-Ser
O18	Fucα1-2Galβ1-4GlcNAcβ1-3Galβ1-3GalNAcα-Ser
O19	Galβ1-4(Fucα1-2)Galβ1-4GlcNAcβ1-3Galβ1-3GalNAcα-Ser
O20	Fucα1-2Galβ1-4(Fucα1-2)Galβ1-4GlcNAcβ1-3Galβ1-3GalNAcα-Ser
O21	Neu5Acα2-6(Neu5Acα2-3(GalNAcβ1-4)Galβ1-3)GalNAcα-Ser
O22	GlcNAcβ1-6(Galβ1-3)GalNAcα-Ser
O23	GlcNAcβ1-6(Galβ1-3)GalNAcα-Thr
O24	Galβ1-4GlcNAcβ1-6(Galβ1-3)GalNAcα-Ser
O25	GlcNAcβ1-3GalNAcα-Ser
O26	GlcNAcβ1-3GalNAcα-Thr
O27	Galβ1-4GlcNAcβ1-3GalNAcα-Ser
O28	Galα1-3Galβ1-4GlcNAcβ1-3GalNAcα-Ser
O29	Neu5Acα2-3Galβ1-4GlcNAcβ1-3GalNAcα-Ser
O30	Neu5Acα2-6Galβ1-4GlcNAcβ1-3GalNAcα-Ser
O31	GalNAcβ1-4(Neu5Acα2-3)Galβ1-4GlcNAcβ1-3GalNAcα-Ser
O32	Galβ1-4(Fucα1-3)GlcNAcβ1-3GalNAcα-Ser
O33	Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-3GalNAcα-Ser
O34	Fucα1-2Galβ1-4GlcNAcβ1-3GalNAcα-Ser
O35	GalNAcα1-3(Fucα1-2)Galb1-4GlcNAcβ1-3GalNAcα
O36	Galα1-3(Fucα1-2)Galb1-4GlcNAcβ1-3GalNAcα
O37	Neu5Acα2-6(GlcNAcβ1-3)GalNAcα-Ser
O38	Neu5Acα2-6(Galβ1-4GlcNAcβ1-3)GalNAcα-Ser
O39	GlcNAcβ1-6(GlcNAcβ1-3)GalNAcα-Thr
O40	GlcNAcβ1-6GalNAcα-Ser
O41	Galβ1-4GlcNAcβ1-6GalNAcα-Ser
O42	Galα1-3Galβ1-4GlcNAcβ1-6GalNAcα-Ser
O43	GalNAcβ1-4(Neu5Acα2-3)Galβ1-4GlcNAcβ1-6GalNAcα-Ser
O44	Galβ1-4(Fucα1-3)GlcNAcβ1-6GalNAcα-Ser
O45	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-6GalNAcα-Ser
O46	Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-6GalNAcα-Ser
O47	Fucα1-2Galβ1-4GlcNAcβ1-6GalNAcα-Ser
O48	GalNAcα 1-3(Fucα1-2)Galβ1-4GlcNAcβ1-6GalNAcα-Ser
O49	GalNAcα-H2N-APGSTAPP-NH2
O50	GalNAcα-H2N-TSAPDTRPAP-NH2

O51	GlcNAcβ1-2Manα-Thr
O52	Galβ1-4GlcNAcβ1-2Manα-Thr
O53	Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα-Thr
O54	Neu5Gcα2-3Galβ1-4GlcNAcβ1-2Manα-Thr
O55	Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα-Thr
O56	Neu5Gcα2-6Galβ1-4GlcNAcβ1-2Manα-Thr
O57	Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα-Thr
O58	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα-Thr
O59	GlcAβ1-3Galβ1-4GlcNAcβ1-2Manα-Thr
O60	GlcNAcβ1-6(GlcNAcβ1-2)Manα-Thr
O61	GlcNAcβ1-6(Galβ1-4GlcNAcβ1-2)Manα-Thr
O62	GlcNAcβ1-6(Neu5Acα2-3Galβ1-4GlcNAcβ1-2)Manα-Thr
O63	GlcNAcβ1-6(Neu5Acα2-6Galβ1-4GlcNAcβ1-2)Manα-Thr
O64	GlcNAcβ1-6(Galβ1-4(Fucα1-3)GlcNAcβ1-2)Manα-Thr
O65	GlcNAcβ1-6(Neu5Acα2-6Galβ1-4(Fucα1-3)GlcNAcβ1-2)Manα-Thr
O66	Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-2)Manα-Thr
O67	Galβ1-4GlcNAcβ1-6(Neu5Acα2-3Galβ1-4GlcNAcβ1-2)Manα-Thr
O68	Galβ1-4GlcNAcβ1-6(Neu5Acα2-6Galβ1-4GlcNAcβ1-2)Manα-Thr
O69	Galβ1-4GlcNAcβ1-6(Galβ1-4(Fucα1-3)GlcNAcβ1-2)Manα-Thr
O70	Galβ1-4GlcNAcβ1-6(Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2)Manα-Thr
O71	Neu5Acα2-3Galβ1-4GlcNAcβ1-6(Neu5Acα2-3Galβ1-4GlcNAcβ1-2)Manα-Thr
O72	Neu5Acα2-3Galβ1-4GlcNAcβ1-6(Neu5Acα2-6Galβ1-4GlcNAcβ1-2)Manα-Thr
O73	Neu5Acα2-3Galβ1-4GlcNAcβ1-6(Galβ1-4(Fucα1-3)GlcNAcβ1-2)Manα-Thr
O74	Neu5Acα2-3Galβ1-4GlcNAcβ1-6(Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2)Manα-Thr
O75	Neu5Acα2-6Galβ1-4GlcNAcβ1-6(Neu5Acα2-6Galβ1-4GlcNAcβ1-2)Manα-Thr
O76	Neu5Acα2-6Galβ1-4GlcNAcβ1-6(Galβ1-4(Fucα1-3)GlcNAcβ1-2)Manα-Thr
O77	Neu5Acα2-6Galβ1-4GlcNAcβ1-6(Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2)Manα-Thr
O78	Galβ1-4(Fucα1-3)GlcNAcβ1-6(Galβ1-4(Fucα1-3)GlcNAcβ1-2)Manα-Thr
O79	Galβ1-4(Fucα1-3)GlcNAcβ1-6(Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2)Manα-Thr
O80	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-6(Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2)Manα-Thr
O81	Galβ1-4GlcNAcβ1-6(GlcNAcβ1-2)Manα-Thr
O82	Neu5Acα2-6Galβ1-4GlcNAcβ1-6(GlcNAcβ1-2)Manα-Thr
O83	Galβ1-4(Fucα1-3)GlcNAcβ1-6(GlcNAcβ1-2)Manα-Thr
O84	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-6(GlcNAcβ1-2)Manα-Thr
O85	Neu5Acα2-3Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-2)Manα-Thr
O86	Neu5Acα2-6Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-2)Manα-Thr
O87	Galβ1-4(Fucα1-3)GlcNAcβ1-6(Galβ1-4GlcNAcβ1-2)Manα
O88	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-6(Galβ1-4GlcNAcβ1-2)Manα-Thr
O89	Neu5Acα2-6Galβ1-4GlcNAcβ1-6(Neu5Acα2-3Galβ1-4GlcNAcβ1-2)Manα-Thr
O90	Galβ1-4(Fucα1-3)GlcNAcβ1-6(Neu5Acα2-3Galβ1-4GlcNAcβ1-2)Manα-Thr
O91	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-6(Neu5Acα2-3Galβ1-4GlcNAcβ1-2)Manα-Thr
O92	Galβ1-4(Fucα1-3)GlcNAcβ1-6(Neu5Acα2-6Galβ1-4GlcNAcβ1-2)Manα-Thr
O93	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-6(Neu5Acα2-6Galβ1-4GlcNAcβ1-2)Manα-Thr
O94	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-6(Galβ1-4(Fucα1-3)GlcNAcβ1-2)Manα-Thr

Controls

NC1: Negative control, Print Buffer for O-Mannose Glycans 51-94

NC2: Negative control, Print Buffer for O-GalNAc Glycans 1-50

PC1: Positive control 1, Biotinylated PEG (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 slide
- 8 or 16 subarray cassette
- Blocking Buffer: NHS Glycan Blocking Buffer (NGBB, Item #10106)
- Glycan Array Assay Buffer (Item #10107)

Materials above are included for purchase in our Microarray Kit (http://www.zbiotech.com/products.html)

- Wash Buffer: 20 mM Tris-HCl, 150 mM NaCl, 0.05% Tween 20, pH 7.6
- Glycan-binding protein samples of interest
- Biotinylated secondary antibodies (for sandwich assay format only)
- Fluorescent labelled streptavidin (for biotin-labelled glycan-binding samples)
- Sterile de-ionized water
- Orbital shaker
- Laser fluorescence scanner (able to scan at the wavelength of your fluorophore)
- Centrifuge
- Pipettors and sterile pipette tips
- Sterile centrifuge tubes
- Coplin jar or 250mL beaker
- Adhesive slide cover film
- Aluminum foil

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 in order 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 120 µL for 8 subarray. 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. 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. 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.
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. 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 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. Analyze data with microarray analysis software. If there is specific binding the signal intensity should be significantly higher than the negative control signal. 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 Controls (Print Buffer): The negative controls should produce little to no signal. Since there is no binding involved with the negative control, any other signals around the negative control's intensity are also not binding.

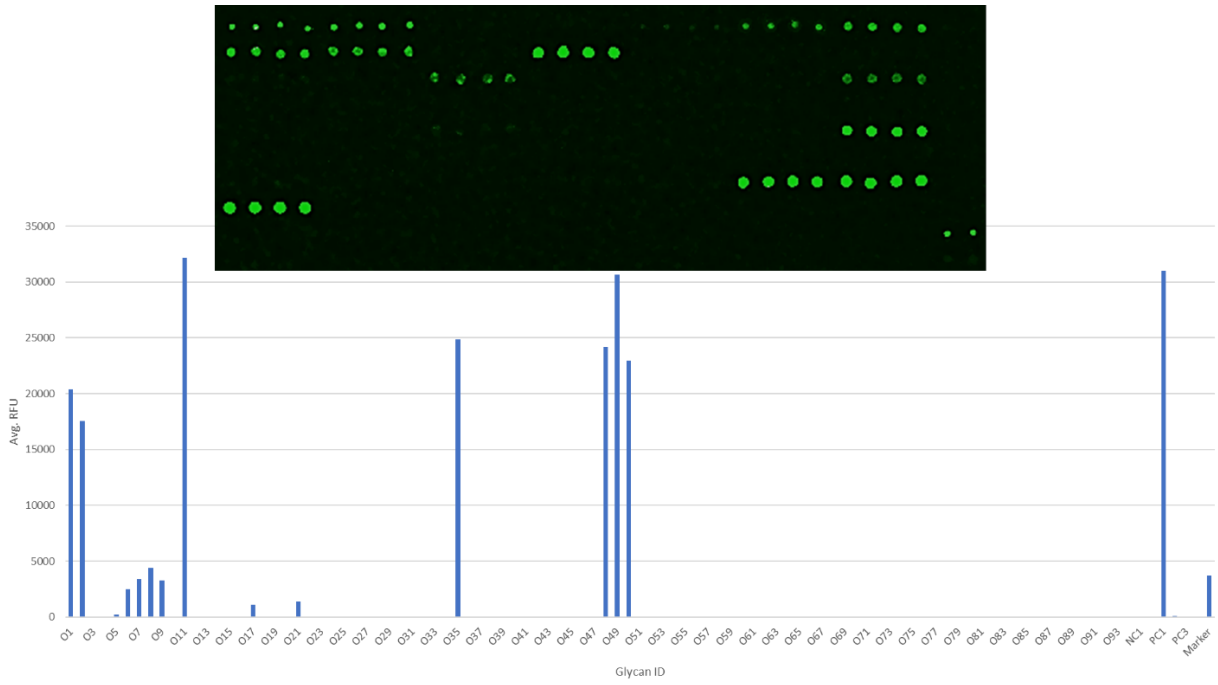
Marker: The array marker should show a strong fluorescence signal regardless of the assay. It is there primarily to aid with orientation of the array map during analysis.

Biotinylated PEG (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 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.

Typical Binding Assay Result from the O-Glycan Array

Example 1: A well on an O-Glycan Array slide (8-subarray) was assayed with glycan-binding protein biotinylated Helix pomatia agglutinin (HPA) lectin (10 μg/mL), followed by Streptavidin-Cy3. Array was scanned with InnoScan 710 microarray scanner at 2 PMT and low laser power at 532nm wavelength. There is very low non-specific binding for the negative control spots. Positive control 1 and the marker show binding as expected, as well as terminal-GalNAc-containing O-glycans.



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