O-Glycan Array User Manual

Z BIOTECH

Innovative Biochemical Analysis Solutions

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

Tel: (720) 285-3587

Email: info@zbiotech.com

For Research Use Only

Introduction

Glycosylation is a post-translational modification in which sugar molecules are covalently attached to the protein, lipid, and nucleic acid. O-linked glycosylation adds an oligosaccharide to the oxygen (O) atom of the hydroxyl group in a serine or threonine residue. This process happens in the endoplasmic reticulum, golgi apparatus, and sometimes in the cytoplasm. O-glycosylated proteins are usually found on the cell surface and in the extracellular matrix, where they play essential roles in tissue structure support, receptor recognition, and signaling regulation.

One of the most famous examples of O-glycosylated proteins is mucin, which coats the mucosal surface of the gastrointestinal and respiratory systems. These proteins are heavily glycosylated with O-glycan structures on serine and threonine residues. Impairment of the O-glycosylation on mucins negatively affects their function. Truncated Mucin O-glycans are manifest in tumor tissues, and these abnormally expressed O-glycans promote self-tolerance and prevent the immune system from attacking the tumor cells. In Alzheimer's disease, several changes in O-glycans of the accumulated tau protein have been detected, which escalates disease progression by promoting neuroinflammation. Identifying and understanding the respective interaction of abnormally expressed O-glycans may unveil novel molecular mechanisms for therapeutic development.

ZBiotech has developed a robust microarray platform that allows researchers to explore the interactions between O-glycans and biological samples such as proteins, antibodies, cells, cell lysate, serum, vesicles, bacteria, or viral particles. The O-glycan array features 94 distinct O-glycan structures. Each array contains 8 or 16 identical subarrays, enabling the simultaneous analysis of multiple samples. The O-Glycan array provides high-throughput and reliable glycan-binding information with a simple assay format that only requires a small sample volume.

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.

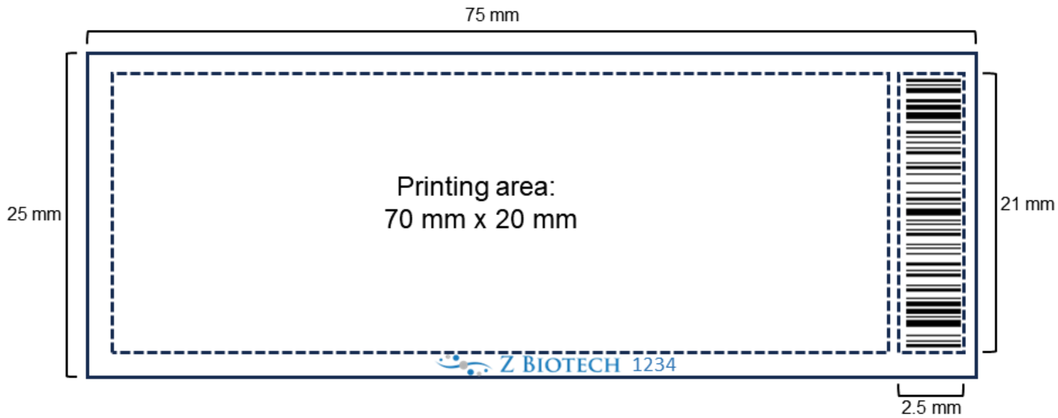
Handling and Storage

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.

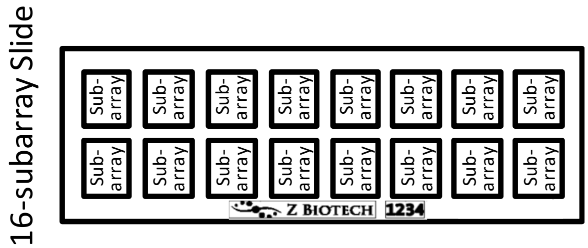
Allow the bag of slides to equilibrate to room temperature at least 20 minutes before opening. After opening, re-seal any unused slides in the moisture barrier bag with a desiccant inside and refreeze.

Array Map/Schematic

O-Glycan Array slides have either 8 or 16 subarrays. Arrays are printed on the side with the "Z Biotech" label and 4-digit number ID facing upward. The "Z Biotech" label is located on the bottom center from a landscape view. The number ID is consistent with the barcode ID on the bottom from a portrait view. Dimensions and array maps are shown below.

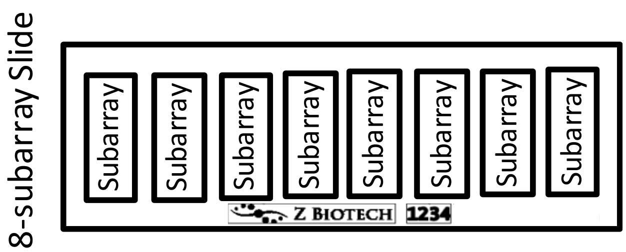

75 mm
25 mm
Printing area:
70 mm x 20 mm
21 mm
Z BIOTECH 1234
2.5 mm

Array Map (16-subarray slides)



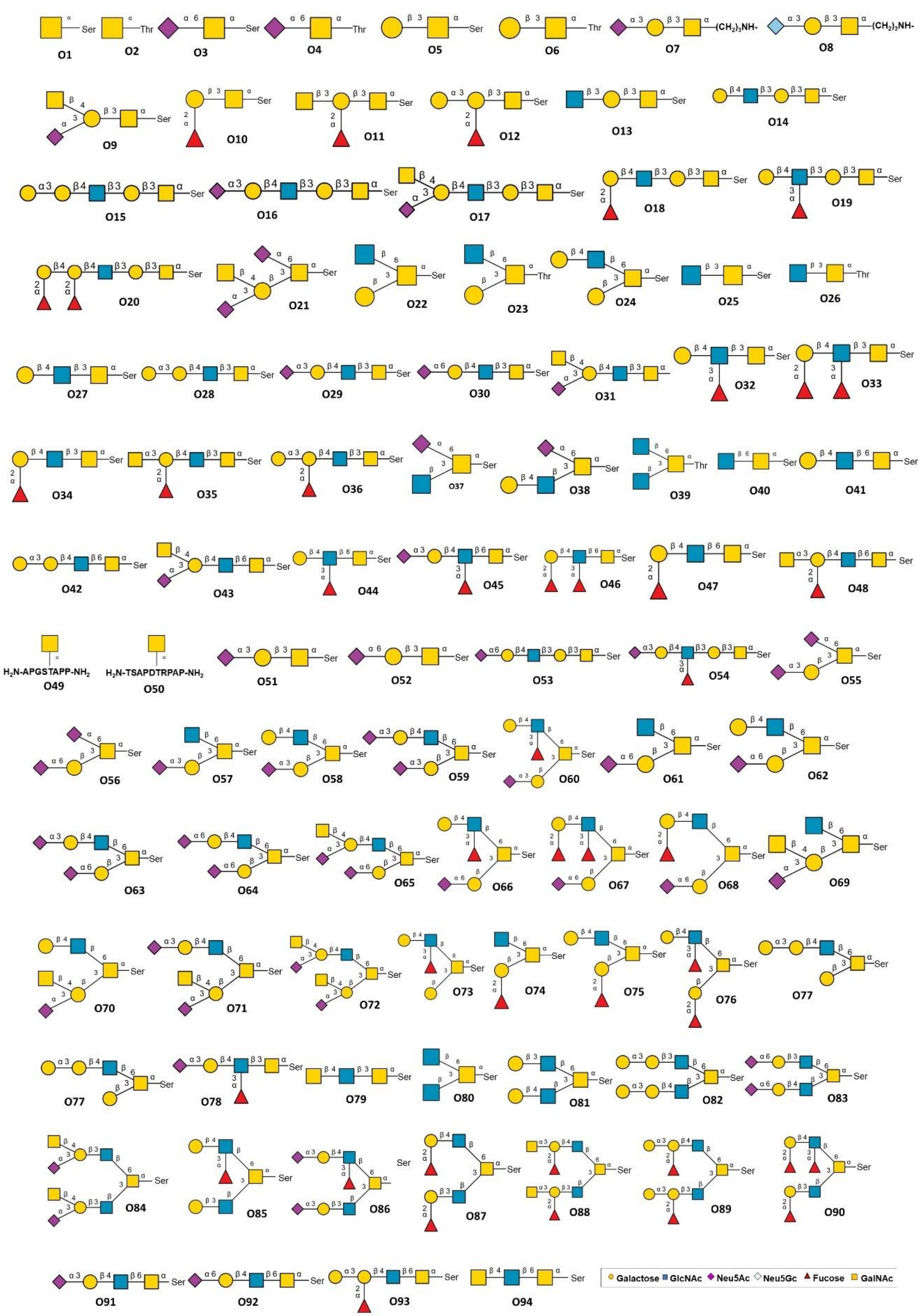
1	1	1	2	2	2	3	3	3	4	4	4	5	5	5	6	6	6
7	7	7	8	8	8	9	9	9	10	10	10	11	11	11	12	12	12
13	13	13	14	14	14	15	15	15	16	16	16	17	17	17	18	18	18
19	19	19	20	20	20	21	21	21	22	22	22	23	23	23	24	24	24
25	25	25	26	26	26	27	27	27	28	28	28	29	29	29	30	30	30
31	31	31	32	32	32	33	33	33	34	34	34	35	35	35	36	36	36
37	37	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	NC	NC	NC	PC1	PC1	PC1
PC2	PC2	PC2	PC3	PC3	PC3	PC4	PC4	PC4	Blank	Blank	Blank	Blank	Blank	Blank	M	M	M

Array Map (8-subarray slides):



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	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	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	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	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	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	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	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	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
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	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	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	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	92	92	92	92	93	93	93	93	94	94	94	94	NC	NC	NC	NC	PC1	PC1	PC1	PC1	PC2	PC2	PC2	PC2	PC3	PC3	PC3	PC3	PC4	PC4	PC4	PC4
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	M	M	M	M

O-Glycan Identification List



ID	Structure
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-(CH2)3NH-
O8	Neu5Gcα2-3Galβ1-3GalNAcα-(CH2)3NH-
O9	Neu5Acα2-3(GalNAcβ1-4)Galβ1-3GalNAcα-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(Fucα1-3)GlcNAcβ1-3Galβ1-3GalNAcα-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-Ser
O36	Galα1-3(Fucα1-2)Galb1-4GlcNAcβ1-3GalNAcα-Ser
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	H2N-APGST*APP-NH2 (*GalNAcα)
O50	H2N-TSAPDT*RPAP-NH2 (*GalNAcα)
O51	Neu5Acα2-3Galβ1-3GalNAcα-Ser
O52	Neu5Acα2-6Galβ1-3GalNAcα-Ser
O53	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-3GalNAcα-Ser
O54	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-3GalNAcα-Ser
O55	Neu5Acα2-3Galβ1-3(Neu5Acα2-6)GalNAcα-Ser
O56	Neu5Acα2-6Galβ1-3(Neu5Acα2-6)GalNAcα-Ser
O57	Neu5Acα2-3Galβ1-3(GlcNAcβ1-6)GalNAcα-Ser
O58	Neu5Acα2-3Galβ1-3(Galβ1-4GlcNAcβ1-6)GalNAcα-Ser
O59	Neu5Acα2-3Galβ1-4GlcNAcβ1-6(Neu5Acα2-3Galβ1-3)GalNAcα-Ser
O60	Galβ1-4(Fucα1-3)GlcNAcβ1-6(Neu5Acα2-3Galβ1-3)GalNAcα-Ser
O61	Neu5Acα2-6Galβ1-3(GlcNAcβ1-6)GalNAcα-Ser
O62	Neu5Acα2-6Galβ1-3(Galβ1-4GlcNAcβ1-6)GalNAcα-Ser
O63	Neu5Acα2-3Galβ1-4GlcNAcβ1-6(Neu5Acα2-6Galβ1-3)GalNAcα-Ser
O64	Neu5Acα2-6Galβ1-4GlcNAcβ1-6(Neu5Acα2-6Galβ1-3)GalNAcα-Ser
O65	Neu5Acα2-3(GalNAcβ1-4)Galβ1-4GlcNAcβ1-6(Neu5Acα2-6Galβ1-3)GalNAcα-Ser
O66	Galβ1-4(Fucα1-3)GlcNAcβ1-6(Neu5Acα2-6Galβ1-3)GalNAcα-Ser
O67	Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-6(Neu5Acα2-6Galβ1-3)GalNAcα-Ser
O68	Fucα1-2Galβ1-4GlcNAcβ1-6(Neu5Acα2-6Galβ1-3)GalNAcα-Ser
O69	Neu5Acα2-3(GalNAcβ1-4)Galβ1-3(GlcNAcβ1-6)GalNAcα-Ser
O70	Neu5Acα2-3(GalNAcβ1-4)Galβ1-3(Galβ1-4GlcNAcβ1-6)GalNAcα-Ser
O71	Neu5Acα2-3(GalNAcβ1-4)Galβ1-3(Neu5Acα2-3Galβ1-4GlcNAcβ1-6)GalNAcα-Ser
O72	Neu5Acα2-3(GalNAcβ1-4)Galβ1-4GlcNAcβ1-6(Neu5Acα2-3(GalNAcβ1-4)Galβ1-3)GalNAcα-Ser
O73	Galβ1-4(Fucα1-3)GlcNAcβ1-6(Galβ1-3)GalNAcα-Ser
O74	Fucα1-2Galβ1-3(GlcNAcβ1-6)GalNAcα-Ser
O75	Fucα1-2Galβ1-3(Galβ1-4GlcNAcβ1-6)GalNAcα-Ser
O76	Galβ1-4(Fucα1-3)GlcNAcβ1-6(Fucα1-2Galβ1-3)GalNAcα-Ser
O77	Galα1-3Galβ1-4GlcNAcβ1-6(Galβ1-3)GalNAcα-Ser
O78	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3GalNAcα-Ser
O79	GalNAcβ1-4GlcNAcβ1-3GalNAcα-Ser
O80	GlcNAcβ1-6(GlcNAcβ1-3)GalNAcα-Ser
O81	Galβ1-4GlcNAcβ1-3(Galβ1-3GlcNAcβ1-6)GalNAcα-Ser
O82	Galα1-3Galβ1-4GlcNAcβ1-3(Galα1-3Galβ1-3GlcNAcβ1-6)GalNAcα-Ser
O83	Neu5Acα2-6Galβ1-4GlcNAcβ1-3(Neu5Acα2-6Galβ1-3GlcNAcβ1-6)GalNAcα-Ser
O84	Neu5Acα2-3(GalNAcβ1-4)Galβ1-3GlcNAcβ1-3(Neu5Acα2-3(GalNAcβ1-4)Galβ1-3GlcNAcβ1-6)GalNAcα-Ser
O85	Galβ1-3(Fucα1-3)GlcNAcβ1-3 (Galβ1-4(Fucα1-3)GlcNAcβ1-6)GalNAcα-Ser
O86	Neu5Acα2-3Galβ1-3(Fucα1-3)GlcNAcβ1-3(Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-6)GalNAcα-Ser
O87	Fucα1-2Galβ1-3GlcNAcβ1-3(Fucα1-2Galβ1-4GlcNAcβ1-6)GalNAcα-Ser
O88	Fucα1-2(GalNAcα1-3)Galβ1-3GlcNAcβ1-3(Fucα1-2(GalNAcα1-3)Galβ1-4GlcNAcβ1-6)GalNAcα-Ser

O89	Fucα1-2(Galα1-3)Galβ1-3GlcNAcβ1-3(Fucα1-2(Galα1-3)Galβ1-4GlcNAcβ1-6)GalNAcα-Ser
O90	Fucα1-2Galβ1-3(Fucα1-3)GlcNAcβ1-3(Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-6)GalNAcα-Ser
O91	Neu5Acα2-3Galβ1-4GlcNAcβ1-6GalNAcα-Ser
O92	Neu5Acα2-6Galβ1-4GlcNAcβ1-6GalNAcα-Ser
O93	Galα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-6GalNAcα-Ser
O94	GalNAcβ1-4GlcNAcβ1-6GalNAcα-Ser

Controls

NC: Negative control, Print Buffer
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 slides
- 16 or 8 cassettes
- Glycan Array Blocking Buffer (GABB, Item #10106), add 1% BSA (10 mg/ml) if needed
- Glycan Array Assay Buffer (GAAB, Item #10107), add 1% BSA (10 mg/ml) if needed
- Laser fluorescence scanner (able to scan at the wavelength of your fluorophore)
- Coplin jar
- Adhesive slide cover film

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

Prepare glycan-binding protein samples or secondary antibodies of interest in a centrifuge tube by diluting with the Glycan Array Assay Buffer. We recommend a range of 50 μg/ml to 0.1 μg/ml concentration for protein samples, although some experimentation may be required to establish the concentration that will provide the highest binding signals with the lowest background fluorescence. This is often accomplished by applying a different dilution of samples to different wells of the array. For the fluorescently labelled streptavidin we recommend a concentration of 1 μg/mL. Calculate the volume of sample needed depending on how many slides and subarrays are to be assayed. **We recommend using 100 µL volume of sample per well for 16 subarray cassettes and 200 µL for 8 subarray cassettes to ensure full and even coverage of the printed area throughout incubation for every step of the assay.** If necessary, the assay can be done successfully with a minimal volume of 60 μL per well for 16 subarray cassettes and 80 μL for 8 subarray cassettes. We caution that using a minimal volume in the wells has an increased risk of the array drying out during the assay and may also cause unequal distribution of the sample across the arrayed surface which may result in signal variation. Please ensure each sample is homogeneous and thoroughly mixed.

Assay Protocol

Part 1 – Blocking

Handle the slide in a clean, dry environment. Use gloves and avoid touching the slide surface.

1. Let the arrayed slides equilibrate to room temperature (20-30 minutes) before opening the moisture barrier bag.
2. Add blocking buffer to each subarray well.
3. Cover the wells with adhesive film to prevent evaporation and incubate slide on shaker at 80 rpm for 30 min. Longer incubation time is acceptable, but not necessary.

Make sure the orbital shaker is completely flat. If the slide is sloped in any direction during incubation, it can cause variation in binding and detection.

Part 2 – Binding Assay

1. Unless the glycan binding protein sample of interest is bacteria or cells, centrifuge samples briefly to avoid adding irrelevant particles to the array.
2. Remove blocking buffer from each well by gently touching a pipette tip to the corner of the well, tipping the slide so that the sample pools to that corner, and pipetting off buffer. Avoid touching the array surface. Have the replacement buffer ready before removing the old buffer to ensure the array does not dry out.
3. Wash the wells three times by adding GAAB to each well and shaking the array at 80 rpm for 5 min. Remove the buffer and repeat.
4. Immediately apply the glycan binding protein sample of interest to each well. Avoid leaving air bubbles.
5. Seal the wells with adhesive film to prevent evaporation. If the sample is fluorescently labelled, cover with aluminum foil to keep it in the dark. Incubate on the shaker for 1 hour at 80 rpm. If the samples can easily aggregate, shake at higher speed to prevent protein aggregation. Longer incubation time may increase binding signal, especially for weakly binding samples.

Avoid allowing the slides to dry out at any point during the assay, especially during long incubation times. Make sure the adhesive film is sealed around each well.

If your glycan-binding protein samples are fluorescently labelled, go directly to Part 6 – Final Wash and Dry.

Part 3 – Wash

1. Remove buffer or sample from each well by gently touching a pipette tip to the corner of the well, tipping the slide so that the sample pools to that corner, and pipetting off buffer. Avoid touching the array surface.
2. Immediately add GAAB to each well. Incubate on the shaker for 5 minutes at 80 rpm. Completely remove the buffer by pipette and repeat this step twice more. Avoid allowing the slide to dry out by having your next wash or sample ready before you remove the buffer.

If your glycan-binding sample is biotinylated, go directly to Part 5 – Fluorescent Staining.

Part 4 – Binding of Biotinylated Antibody (Sandwich Assay Format)

1. Unless the secondary biotinylated antibody sample is bacteria or cells, centrifuge samples briefly to avoid adding irrelevant particles to the array.
2. After completely removing the third GAAB wash, immediately add the secondary biotinylated antibody to each well. Seal the wells with adhesive film and incubate on the shaker for 1 hour at 80 rpm. Shaking at a faster speed can prevent protein aggregation. Longer incubation time is acceptable, but not necessary.

3. After incubation repeat Part 3 – Wash.

Part 5 – Fluorescent Staining

1. Centrifuge fluorescent labeled streptavidin samples briefly to avoid adding irrelevant particles to the array.
2. After completely removing the third GAAB wash, immediately add the fluorescently labelled streptavidin sample. Seal the wells with adhesive film and shield the wells from light with aluminum foil. Incubate on the shaker at 80 rpm for 1 hour. Longer incubation time is acceptable, but not necessary.

Part 6 – Final Wash and Dry

1. Remove sample from each well by gently touching a pipette tip to the corner of the well, tipping the slide so that the liquid pools to that corner, and pipetting off. Avoid touching the array surface.
2. Briefly rinse each well with GAAB.
3. Completely remove the buffer by pipette. Avoid touching the array surface. Repeat steps 2 and 3.
4. Disassemble the cassette from the slide. For the provided cassette this can be done by holding the slide with one hand at the top and bottom edges and sliding out the cassette clips one by one with the other hand. If your provided cassette has metal clips, they can be removed by rotating the clip outwards from the bottom of the slide. When the clips have been removed place the slide on the table and hold a small outer edge of the slide to the table as you gently peel the cassette off.
5. Immediately immerse the slide in a coplin jar or beaker full of GAAB. Do not touch the surface of the array or allow the array surface to touch the sides of the beaker or jar.
6. Place the jar or beaker on a shaker at 80 rpm for 10 minutes.
7. Decant the buffer from the jar or beaker while holding the slide in place (only touch the edge of the slide) and then add sterile de-ionized water to immerse the slide.
8. Place the jar or beaker on the shaker at 80 rpm for 2 minutes.
9. Decant the water from the jar or beaker. Repeat once more with fresh de-ionized water.
10. Allow the slide to dry completely in a clean, dust free environment before scanning.

Analysis

Scan the slide in a laser fluorescence scanner at the wavelength of emission for the fluorophore used. Adjust the laser power and PMT to obtain the highest possible signals without any being saturated (saturated positive control signal is okay). Analyze data with microarray analysis software. If there is specific binding the signal intensity should be higher than the background signal (area where there are no printed spots). Fluorescent signal due to specific binding to your sample of interest should be both dose-dependent with your sample dilution (unless the sample concentration range is too high and glycan binding is saturated) and should have positive binding signal after signal from control assays has been subtracted. Our standard method of comparing signal intensities is to quantify the median signal intensity data and subtract the background intensity. Subtracting signal from negative control spots as well as the same spots on a negative control assay (assay with only detection antibodies and fluorophore) will give more accurate specific binding data.

Interpretation of Control Signals:

Negative 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.

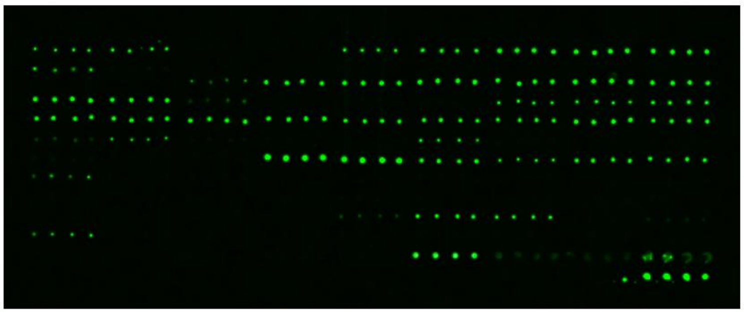
Marker: The array marker should show a strong fluorescence signal regardless of the assay. It is there primarily to aid with orientation of the array map during analysis.

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.

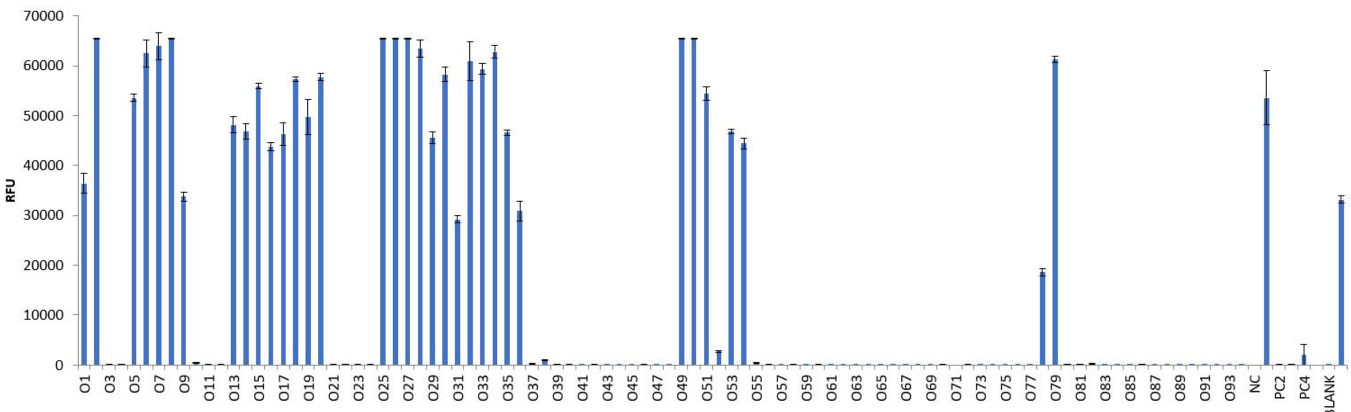
Typical Binding Assay Result from the O-Glycan Array

The O-glycan array was assayed with biotinylated Artocarpus integrifolia Lectin (AIA) (10 µg/mL), followed by streptavidin (Cy3). The array was scanned with a microarray scanner at 532nm wavelength. Positive control showed binding signals as expected. AIA binds to terminal GalNAc-containing O-glycans.



Array Scan Image

The microarray slide was scanned by a microarray slide scanner at high laser intensity.



Quantitative Data

Data was generated by analyzing scanned microarray images.

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