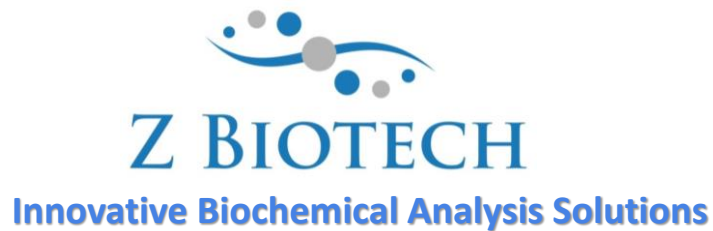


# O-Glycan Array User Manual



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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.

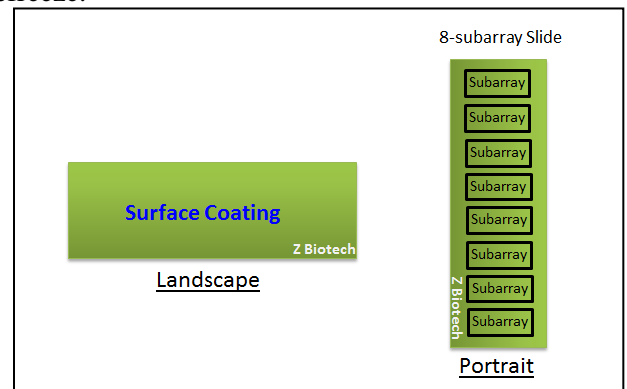
## Handling and Storage

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.

## Array Map/Schematic

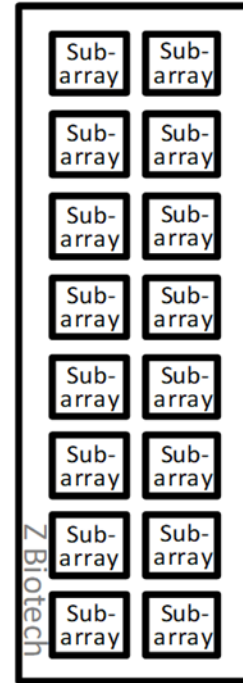
O-Glycan Array slides have either 8 or 16 subarrays. Arrays are printed on the side with the “Z Biotech” label facing upward. The “Z Biotech” label is located on the bottom right corner from a landscape point of view, or the bottom left corner in a portrait point of view (see image on right). The orientation 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:



**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	NC	NC	NC	PC1	PC1	PC1
17	PC2	PC2	PC2	PC3	PC3	PC3	PC4	PC4	PC4	Blank	Blank	Blank	Blank	Blank	Blank	M	M	M

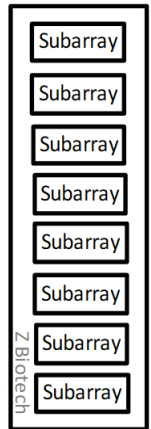
**16-subarray Slide**



**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

**8-subarray Slide**



## O-Glycan Identification List

ID	Structure
O1	GalNAc $\alpha$
O2	GalNAc $\alpha$
O3	Neu5Ac $\alpha$ 2-6GalNAc $\alpha$
O4	Neu5Ac $\alpha$ 2-6GalNAc $\alpha$
O5	Gal $\beta$ 1-3GalNAc $\alpha$
O6	Gal $\beta$ 1-3GalNAc $\alpha$
O7	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GalNAc $\alpha$
O8	Neu5Gc $\alpha$ 2-3Gal $\beta$ 1-3GalNAc $\alpha$
O9	GalNAc $\beta$ 1-4(Neu5Ac $\alpha$ 2-3)GalNAc $\alpha$
O10	Fuc $\alpha$ 1-2Gal $\beta$ 1-3GalNAc $\alpha$
O11	GalNAc $\beta$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-3GalNAc $\alpha$
O12	Gal $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-3GalNAc $\alpha$
O13	GlcNAc $\beta$ 1-3Gal $\beta$ 1-3GalNAc $\alpha$
O14	Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-3GalNAc $\alpha$
O15	Gal $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-3GalNAc $\alpha$
O16	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-3GalNAc $\alpha$
O17	GalNAc $\beta$ 1-4(Neu5Ac $\alpha$ 2-3)Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-3GalNAc $\alpha$
O18	Fuc $\alpha$ 1-2Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-3GalNAc $\alpha$
O19	Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-3Gal $\beta$ 1-3GalNAc $\alpha$
O20	Fuc $\alpha$ 1-2Gal $\beta$ 1-4(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-3GalNAc $\alpha$
O21	Neu5Ac $\alpha$ 2-6(Neu5Ac $\alpha$ 2-3(GalNAc $\beta$ 1-4)Gal $\beta$ 1-3)GalNAc $\alpha$
O22	GlcNAc $\beta$ 1-6(Gal $\beta$ 1-3)GalNAc $\alpha$
O23	GlcNAc $\beta$ 1-6(Gal $\beta$ 1-3)GalNAc $\alpha$
O24	Gal $\beta$ 1-4GlcNAc $\beta$ 1-6(Gal $\beta$ 1-3)GalNAc $\alpha$
O25	GlcNAc $\beta$ 1-3GalNAc $\alpha$
O26	GlcNAc $\beta$ 1-3GalNAc $\alpha$
O27	Gal $\beta$ 1-4GlcNAc $\beta$ 1-3GalNAc $\alpha$
O28	Gal $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3GalNAc $\alpha$
O29	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3GalNAc $\alpha$
O30	Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-3GalNAc $\alpha$
O31	GalNAc $\beta$ 1-4(Neu5Ac $\alpha$ 2-3)Gal $\beta$ 1-4GlcNAc $\beta$ 1-3GalNAc $\alpha$
O32	Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-3GalNAc $\alpha$
O33	Fuc $\alpha$ 1-2Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-3GalNAc $\alpha$
O34	Fuc $\alpha$ 1-2Gal $\beta$ 1-4GlcNAc $\beta$ 1-3GalNAc $\alpha$
O35	GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4GlcNAc $\beta$ 1-3GalNAc $\alpha$
O36	Gal $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4GlcNAc $\beta$ 1-3GalNAc $\alpha$
O37	Neu5Ac $\alpha$ 2-6(GlcNAc $\beta$ 1-3)GalNAc $\alpha$
O38	Neu5Ac $\alpha$ 2-6(Gal $\beta$ 1-4GlcNAc $\beta$ 1-3)GalNAc $\alpha$
O39	GlcNAc $\beta$ 1-6(GlcNAc $\beta$ 1-3)GalNAc $\alpha$
O40	GlcNAc $\beta$ 1-6GalNAc $\alpha$
O41	Gal $\beta$ 1-4GlcNAc $\beta$ 1-6GalNAc $\alpha$
O42	Gal $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-6GalNAc $\alpha$
O43	GalNAc $\beta$ 1-4(Neu5Ac $\alpha$ 2-3)Gal $\beta$ 1-4GlcNAc $\beta$ 1-6GalNAc $\alpha$
O44	Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-6GalNAc $\alpha$
O45	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-6GalNAc $\alpha$
O46	Fuc $\alpha$ 1-2Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-6GalNAc $\alpha$
O47	Fuc $\alpha$ 1-2Gal $\beta$ 1-4GlcNAc $\beta$ 1-6GalNAc $\alpha$
O48	GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4GlcNAc $\beta$ 1-6GalNAc $\alpha$
O49	GalNAc $\alpha$ -H2N-APGSTAPP-NH2
O50	GalNAc $\alpha$ -H2N-TSAPDTRPAP-NH2
O51	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GalNAc $\alpha$
O52	Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-3GalNAc $\alpha$
O53	Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-3GalNAc $\alpha$
O54	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-3Gal $\beta$ 1-3GalNAc $\alpha$
O55	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3(Neu5Ac $\alpha$ 2-6)GalNAc $\alpha$
O56	Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-3(Neu5Ac $\alpha$ 2-6)GalNAc $\alpha$
O57	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3(GlcNAc $\beta$ 1-6)GalNAc $\alpha$
O58	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3(Gal $\beta$ 1-4GlcNAc $\beta$ 1-6)GalNAc $\alpha$
O59	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-6(Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3)GalNAc $\alpha$
O60	Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-6(Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3)GalNAc $\alpha$
O61	Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-3(GlcNAc $\beta$ 1-6)GalNAc $\alpha$
O62	Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-3(Gal $\beta$ 1-4GlcNAc $\beta$ 1-6)GalNAc $\alpha$
O63	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-6(Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-3)GalNAc $\alpha$

O64	Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-6(Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-3)GalNAc $\alpha$
O65	Neu5Ac $\alpha$ 2-3(GalNAc $\beta$ 1-4)Gal $\beta$ 1-4GlcNAc $\beta$ 1-6(Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-3)GalNAc $\alpha$
O66	Gal $\beta$ 1-4(Fuca1-3)GlcNAc $\beta$ 1-6(Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-3)GalNAc $\alpha$
O67	Fuca1-2Gal $\beta$ 1-4(Fuca1-3)GlcNAc $\beta$ 1-6(Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-3)GalNAc $\alpha$
O68	Fuca1-2Gal $\beta$ 1-4GlcNAc $\beta$ 1-6(Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-3)GalNAc $\alpha$
O69	Neu5Ac $\alpha$ 2-3(GalNAc $\beta$ 1-4)Gal $\beta$ 1-3(GlcNAc $\beta$ 1-6)GalNAc $\alpha$
O70	Neu5Ac $\alpha$ 2-3(GalNAc $\beta$ 1-4)Gal $\beta$ 1-3(Gal $\beta$ 1-4GlcNAc $\beta$ 1-6)GalNAc $\alpha$
O71	Neu5Ac $\alpha$ 2-3(GalNAc $\beta$ 1-4)Gal $\beta$ 1-3(Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-6)GalNAc $\alpha$
O72	Neu5Ac $\alpha$ 2-3(GalNAc $\beta$ 1-4)Gal $\beta$ 1-4GlcNAc $\beta$ 1-6(Neu5Ac $\alpha$ 2-3(GalNAc $\beta$ 1-4)Gal $\beta$ 1-3)GalNAc $\alpha$
O73	Gal $\beta$ 1-4(Fuca1-3)GlcNAc $\beta$ 1-6(Gal $\beta$ 1-3)GalNAc $\alpha$
O74	Fuca1-2Gal $\beta$ 1-3(GlcNAc $\beta$ 1-6)GalNAc $\alpha$
O75	Fuca1-2Gal $\beta$ 1-3(Gal $\beta$ 1-4GlcNAc $\beta$ 1-6)GalNAc $\alpha$
O76	Gal $\beta$ 1-4(Fuca1-3)GlcNAc $\beta$ 1-6(Fuca1-2Gal $\beta$ 1-3)GalNAc $\alpha$
O77	Gal $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-6(Gal $\beta$ 1-3)GalNAc $\alpha$
O78	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4(Fuca1-3)GlcNAc $\beta$ 1-3GalNAc $\alpha$
O79	GalNAc $\beta$ 1-4GlcNAc $\beta$ 1-3GalNAc $\alpha$
O80	GlcNAc $\beta$ 1-6(GlcNAc $\beta$ 1-3)GalNAc $\alpha$
O81	Gal $\beta$ 1-4GlcNAc $\beta$ 1-3(Gal $\beta$ 1-3GlcNAc $\beta$ 1-6)GalNAc $\alpha$
O82	Gal $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3(Gal $\alpha$ 1-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-6)GalNAc $\alpha$
O83	Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-3(Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-3GlcNAc $\beta$ 1-6)GalNAc $\alpha$
O84	Neu5Ac $\alpha$ 2-3(GalNAc $\beta$ 1-4)Gal $\beta$ 1-3GlcNAc $\beta$ 1-3(Neu5Ac $\alpha$ 2-3(GalNAc $\beta$ 1-4)Gal $\beta$ 1-3GlcNAc $\beta$ 1-6)GalNAc $\alpha$
O85	Gal $\beta$ 1-3(Fuca1-3)GlcNAc $\beta$ 1-3 (Gal $\beta$ 1-4(Fuca1-3)GlcNAc $\beta$ 1-6)GalNAc $\alpha$
O86	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3(Fuca1-3)GlcNAc $\beta$ 1-3(Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4(Fuca1-3)GlcNAc $\beta$ 1-6)GalNAc $\alpha$
O87	Fuca1-2Gal $\beta$ 1-3GlcNAc $\beta$ 1-3(Fuca1-2Gal $\beta$ 1-4GlcNAc $\beta$ 1-6)GalNAc $\alpha$
O88	Fuca1-2(GalNAc $\alpha$ 1-3)Gal $\beta$ 1-3GlcNAc $\beta$ 1-3(Fuca1-2(GalNAc $\alpha$ 1-3)Gal $\beta$ 1-4GlcNAc $\beta$ 1-6)GalNAc $\alpha$
O89	Fuca1-2(Gal $\alpha$ 1-3)Gal $\beta$ 1-3GlcNAc $\beta$ 1-3(Fuca1-2(Gal $\alpha$ 1-3)Gal $\beta$ 1-4GlcNAc $\beta$ 1-6)GalNAc $\alpha$
O90	Fuca1-2Gal $\beta$ 1-3(Fuca1-3)GlcNAc $\beta$ 1-3(Fuca1-2Gal $\beta$ 1-4(Fuca1-3)GlcNAc $\beta$ 1-6)GalNAc $\alpha$
O91	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-6GalNAc $\alpha$
O92	Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-6GalNAc $\alpha$
O93	Gal $\alpha$ 1-3(Fuca1-2)Gal $\beta$ 1-4GlcNAc $\beta$ 1-6GalNAc $\alpha$
O94	GalNAc $\beta$ 1-4GlcNAc $\beta$ 1-6GalNAc $\alpha$

## **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
- Wash Buffer: 50 mM Tris-HCl, 137 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 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  $\mu$ g/ml to 0.1  $\mu$ g/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 fluorescently labelled streptavidin we suggest a concentration of 1  $\mu$ g/mL. Calculate the volume of sample needed depending on how many slides and subarrays are to be assayed. Use 100  $\mu$ L volume of sample per well for 16

subarray cassettes and 200  $\mu\text{L}$  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  $\mu\text{L}$  per well for 16 subarray cassettes and 120  $\mu\text{L}$  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.

## **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  $\mu\text{L}$  per well for 16 subarray cassettes and 200  $\mu\text{L}$  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 and remove blocking buffer. Avoid touching the array surface.
3. Immediately apply the glycan-binding sample of interest to each well. We recommend using 100  $\mu\text{L}$  per well for 16 subarray cassettes and 200  $\mu\text{L}$  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  $\mu\text{L}$  per well for 16 subarray cassettes and 200  $\mu\text{L}$  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  $\mu\text{L}$  per well for 16 subarray cassettes and 200  $\mu\text{L}$  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  $\mu\text{L}$  per well is recommended for 16 subarray cassettes and 200  $\mu\text{L}$  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. 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. Briefly rinse each well with wash buffer. 100  $\mu\text{L}$  per well is recommended for 16 subarray cassettes and 200  $\mu\text{L}$  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.

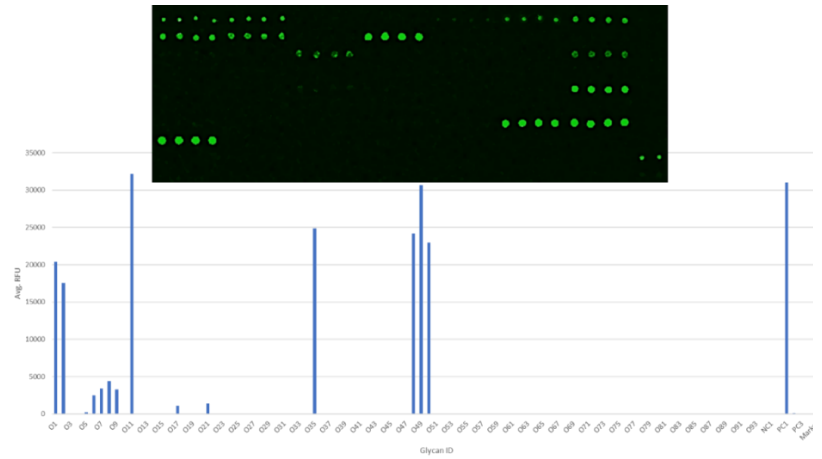
Marker: The array marker should show a strong fluorescence signal regardless of the assay. It is there primarily to aid with orientation of the array map during analysis.

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 performed (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  $\mu\text{g}/\text{mL}$ ), 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	<ul style="list-style-type: none"> <li>• Concentration of glycan-binding protein samples is too high</li> <li>• Concentration of fluorescent samples is too high.</li> <li>• Arrays are not thoroughly washed</li> <li>• Slide drying out during assay</li> <li>• Excessive particles in the samples due to sample aggregation, dust, etc.</li> </ul>	<ul style="list-style-type: none"> <li>• Use a lower concentration range of samples. Consider a wider range if you are unsure where the detection limit is.</li> <li>• Apply longer times for washing steps and use a higher shaking rate</li> <li>• Make sure wash buffer and sample is completely removed before the next step</li> <li>• Make sure adhesive film fully seals the wells to avoid evaporation</li> <li>• Centrifuge the samples prior to assay to avoid adding irrelevant particles</li> <li>• If you think that the protein is aggregating during incubation, try shaking at a higher speed</li> </ul>
Signal Variation	<ul style="list-style-type: none"> <li>• Slide drying out during assay.</li> <li>• Binding samples are not equally distributed in the wells</li> <li>• Glycan-binding protein aggregation during incubation</li> <li>• Bubbles during incubation</li> </ul>	<ul style="list-style-type: none"> <li>• Make sure wells are sealed to prevent evaporation during incubation.</li> <li>• Apply a larger volume of sample to each well to ensure equal distribution</li> <li>• Use a higher shaking rate during incubation</li> <li>• Make sure samples are homogeneous, mixed thoroughly, and do not leave bubbles on the array surface</li> </ul>
Unexpected Binding	<ul style="list-style-type: none"> <li>• Cross contamination between wells or other sources.</li> <li>• Sample contamination</li> </ul>	<ul style="list-style-type: none"> <li>• Make sure to use sterilized pipette tips and tubes used for sample application and preparation</li> <li>• Ensure cassette is pressed firmly to the slide so that there are no gaps to allow leaking between wells</li> <li>• Be careful not to cross contaminate samples when applying to the wells, even during wash steps</li> </ul>