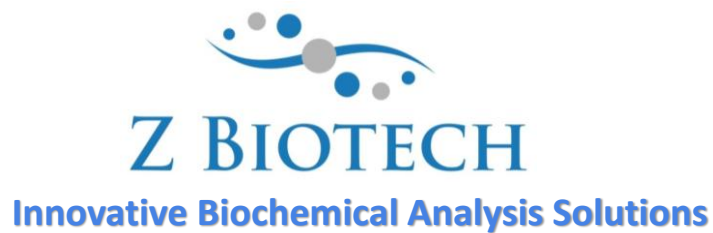


Tumor Associated Glycan Microarray User Manual



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Introduction

The Tumour-Associated Glycan (TAG) Array is developed to help researchers explore interactions between cancer-associated glycans and glycan-binding molecules. The array contains 84 structurally defined tumour-associated glycans and glycopeptides representing key glycan motifs frequently altered in cancer, including truncated O-glycans (Tn, STn, T, and ST antigens), mucin-derived glycopeptides (MUC1 and MUC16 epitopes), sialyl-Lewis antigens associated with CA19-9, gangliosides, Globo-H, SSEA antigens, Neu5Gc-containing glycans, sTRA structures, and cancer associated complex N-glycans. These structures collectively capture the diversity of glycan epitopes implicated in tumor progression, immune recognition, and cancer diagnostics.

The array is designed for high-resolution characterization of glycan-binding specificity and can be used with a wide range of biological samples, including antibodies, lectins, recombinant proteins, serum samples, cell lysates, extracellular vesicles, bacteria, and viral particles. Multiple identical subarrays are created on each slide, enabling parallel analysis of several samples in a single experiment while requiring only minimal sample volume. The platform offers a simple experimental workflow and produces reproducible, high-throughput binding data suitable for both exploratory research and translational studies.

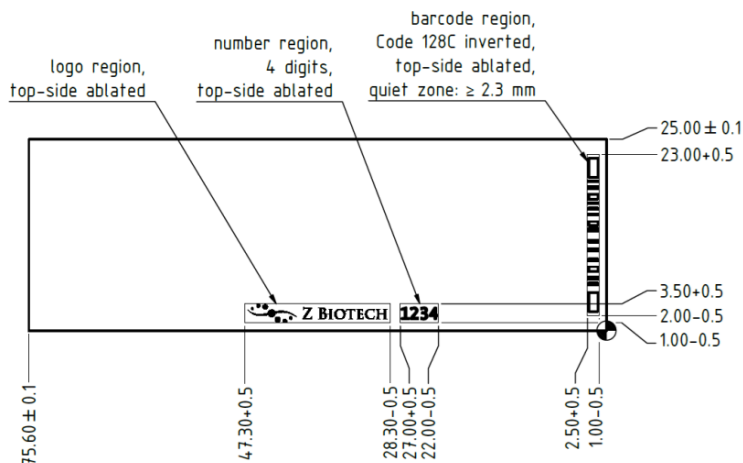
Handling and Storage

Store the bag of slides and any buffers in a 4°C refrigerator if they are to be assayed within 3 weeks upon receipt. For long term storage keep the bag of slides at -20°C. Avoid freezing and thawing multiple times. Purchased slides and buffers should be used within 6 months.

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

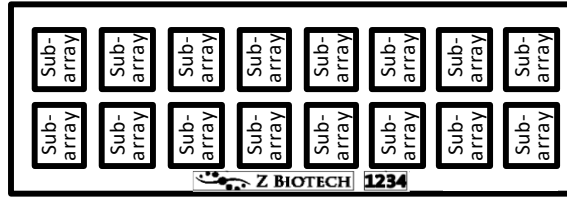
Array Map/Schematic

Tumor Associated Glycan Microarray slides have 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.



Array Map (16-subarray slides):

16-subarray Slide



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
NC	NC	NC	PC1	PC1	PC1	PC2	PC2	PC2	PC3	PC3	PC3	PC4	PC4	PC4	M	M	M

Tumor Associated Glycan Array SNFG Structures:

Pre-TACA's

T1

T2

T3

T4



AFP-L3

T5

T6

T7

T8



CA15-3 (Muc1)

T9

T10

T11

T12

PPAHGVTSAPDTRPAPGSTAPPA PPAHGVTSAPDTRPAPGSTAPPA PPAHGVTSAPDTRPAPGSTAPPA PPAHGVTSAPDTRPAPGSTAPPA

T13

T14

T15

T16

PPAHGVTSAPDTRPAPGSTAPPA PPAHGVTSAPDTRPAPGSTAPPA PPAHGVTSAPDTRPAPGSTAPPA PPAHGVTSAPDTRPAPGSTAPPA

T17

T18

T19

T20

PPAHGVTSAPDTRPAPGSTAPPA PPAHGVTSAPDTRPAPGSTAPPA PPAHGVTSAPDTRPAPGSTAPPA PPAHGVTSAPDTRPAPGSTAPPA

T21

T22

T23

T24

PPAHGVTSAPDTRPAPGSTAPPA PPAHGVTSAPDTRPAPGSTAPPA PPAHGVTSAPDTRPAPGSTAPPA PPAHGVTSAPDTRPAPGSTAPPA

T25

T26

T27

T28

PPAHGVTSAPDTRPAPGSTAPPA PPAHGVTSAPDTRPAPGSTAPPA PPAHGVTSAPDTRPAPGSTAPPA PPAHGVTSAPDTRPAPGSTAPPA

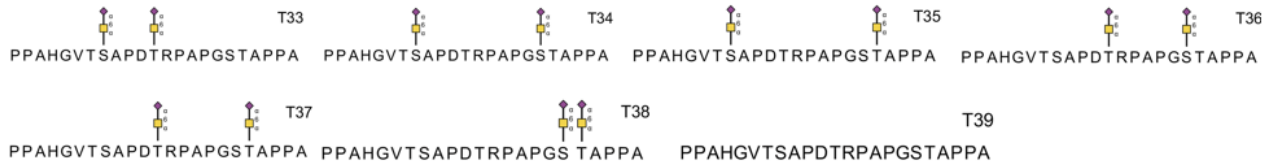
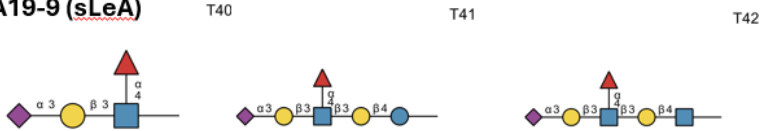
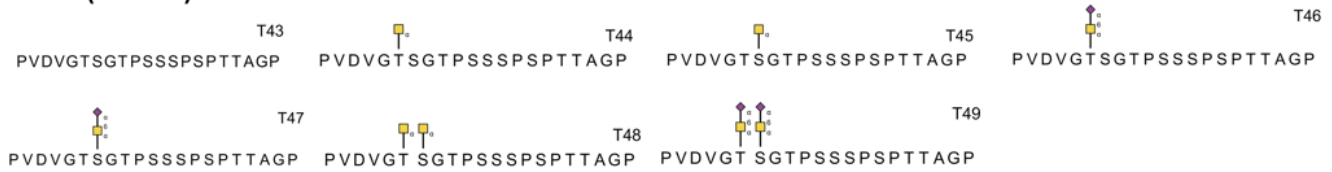
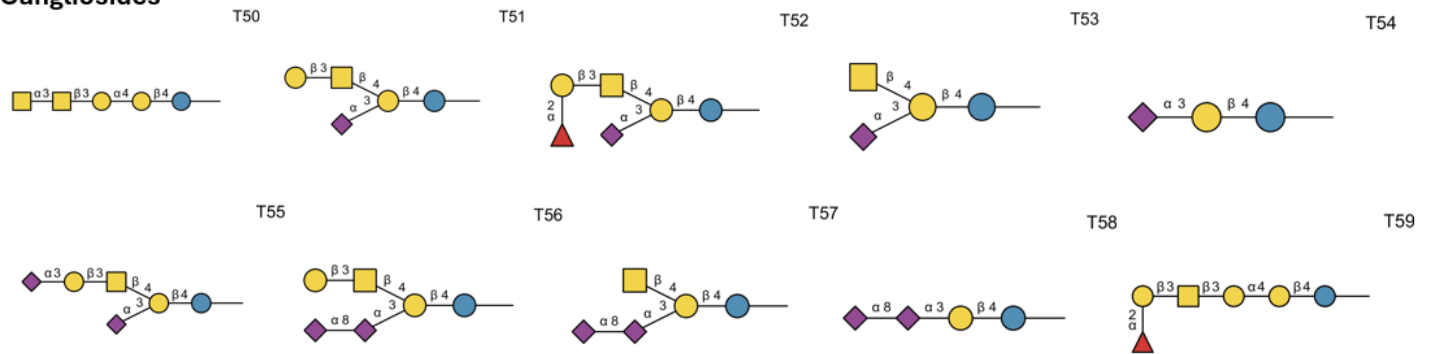
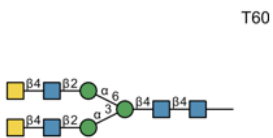
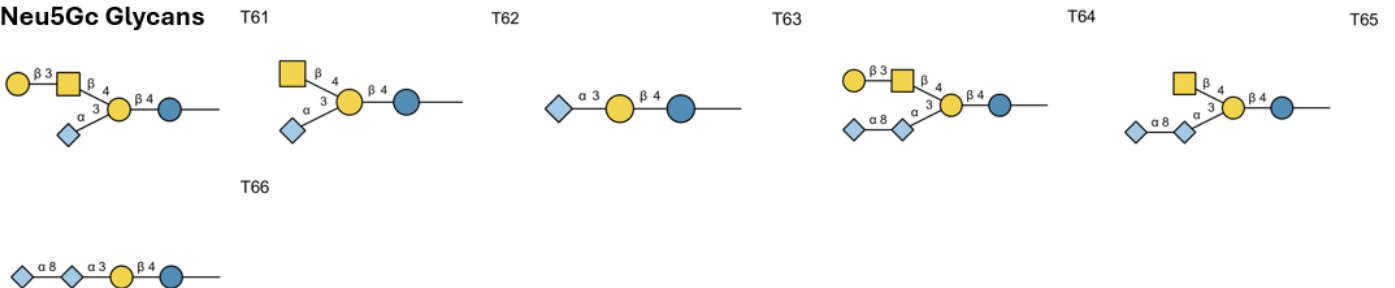
T29

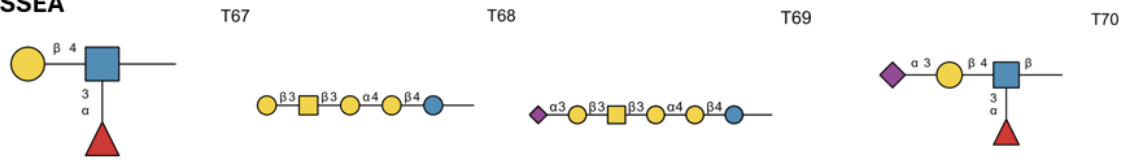
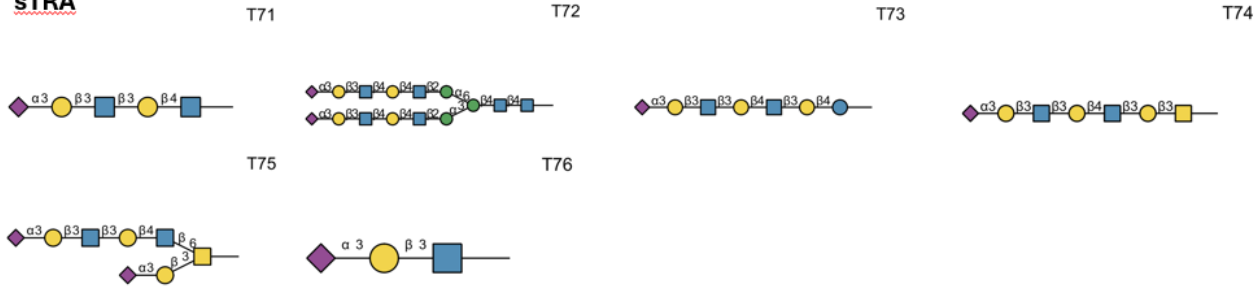
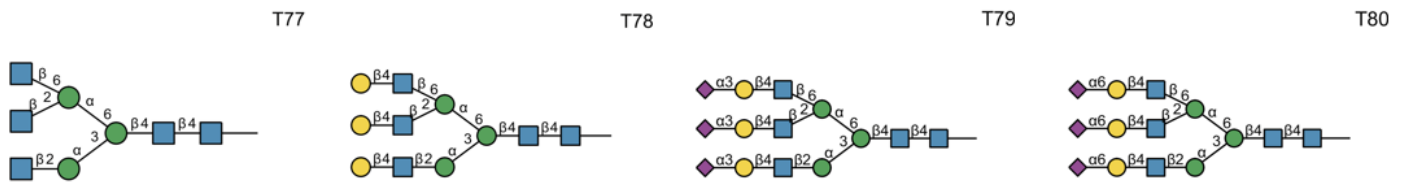
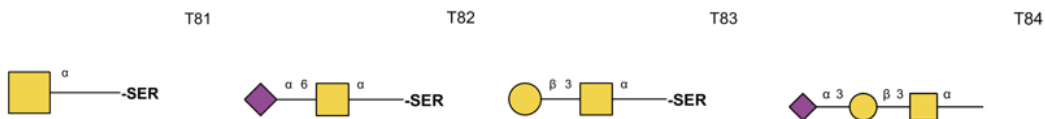
T30

T31

T32

PPAHGVTSAPDTRPAPGSTAPPA PPAHGVTSAPDTRPAPGSTAPPA PPAHGVTSAPDTRPAPGSTAPPA PPAHGVTSAPDTRPAPGSTAPPA

**CA19-9 (sLeA)****CA125 (MUC16)****Gangliosides****LacdiNAc****Neu5Gc Glycans**

SSEA**sTRA****Tri-antennary N-glycan with $\beta 6$ -GlcNAc branch****Tn, sTn, T, and sT antigens**

Identification List:

TAG Class	ID	Name	Text Structure
Pre-TACAs	T1	LNT	Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc
	T2	LNnT	Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc
	T3	LsTa	Neu5Aca2-3Gal β 1-3GlcNAc β 1-3Gal β 1-4Glc
	T4	A3LNnT	Neu5Aca2-3Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc
AFP-L3	T5	G0F	GlcNAc β 1-2Man α 1-6(GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4(Fuca1-6)GlcNAc-
	T6	G2F	Gal β 1-4GlcNAc β 1-2Man α 1-6(Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4(Fuca1-6)GlcNAc-
	T7	G2S2(3)F	Neu5Aca2-3Gal β 1-4GlcNAc β 1-2Man α 1-6(Neu5Aca2-3Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4(Fuca1-6)GlcNAc-
	T8	G2S2(6)F	Neu5Aca2-6Gal β 1-4GlcNAc β 1-2Man α 1-6(Neu5Aca2-6Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4(Fuca1-6)GlcNAc-
CA15-3 (MUC1)	T9	MUC1	NH2-PPAHGV{GalNAc}TSAPDTRPAPGSTAPPA-OH
	T10	MUC2	NH2-PPAHGVT{GalNAc}SAPDTRPAPGSTAPPA-OH
	T11	MUC3	NH2-PPAHGVTSAPD{GalNAc}TRPAPGSTAPPA-OH
	T12	MUC4	NH2-PPAHGVTSAPDTRPAPG{GalNAc}STAPPA-OH
	T13	MUC5	NH2-PPAHGVTSAPDTRPAPGS{GalNAc}TAPPA-OH
	T14	MUC6	NH2-PPAHGV{GalNAc}T{GalNAc}SAPDTRPAPGSTAPPA-OH
	T15	MUC7	NH2-PPAHGV{GalNAc}TSAPD{GalNAc}TRPAPGSTAPPA-OH
	T16	MUC8	NH2-PPAHGV{GalNAc}TSAPDTRPAPG{GalNAc}STAPPA-OH
	T17	MUC9	NH2-PPAHGV{GalNAc}TSAPDTRPAPGS{GalNAc}TAPPA-OH
	T18	MUC10	NH2-PPAHGVT{GalNAc}SAPD{GalNAc}TRPAPGSTAPPA-OH
	T19	MUC11	NH2-PPAHGVT{GalNAc}SAPDTRPAPG{GalNAc}STAPPA-OH
	T20	MUC12	NH2-PPAHGVT{GalNAc}SAPDTRPAPGS{GalNAc}TAPPA-OH
	T21	MUC13	NH2-PPAHGVTSAPD{GalNAc}TRPAPG{GalNAc}STAPPA-OH
	T22	MUC14	NH2-PPAHGVTSAPD{GalNAc}TRPAPGS{GalNAc}TAPPA-OH
	T23	MUC15	NH2-PPAHGVTSAPDTRPAPG{GalNAc}S{GalNAc}TAPPA-OH
	T24	MUC32	NH2-PPAHGV{Neu5Aca2-6GalNAc}TSAPDTRPAPGSTAPPA-OH
	T25	MUC33	NH2-PPAHGVT{Neu5Aca2-6GalNAc}SAPDTRPAPGSTAPPA-OH
	T26	MUC34	NH2-PPAHGVTSAPD{Neu5Aca2-6GalNAc}TRPAPGSTAPPA-OH
	T27	MUC35	NH2-PPAHGVTSAPDTRPAPG{Neu5Aca2-6GalNAc}STAPPA-OH
	T28	MUC36	NH2-PPAHGVTSAPDTRPAPGS{Neu5Aca2-6GalNAc}TAPPA-OH
	T29	MUC37	NH2-PPAHGV{Neu5Aca2-6GalNAc}T{Neu5Aca2-6GalNAc}SAPDTRPAPGSTAPPA-OH
	T30	MUC38	NH2-PPAHGV{Neu5Aca2-6GalNAc}TSAPD{Neu5Aca2-6GalNAc}TRPAPGSTAPPA-OH
	T31	MUC39	NH2-PPAHGV{Neu5Aca2-6GalNAc}TSAPDTRPAPG{Neu5Aca2-6GalNAc}STAPPA-OH
	T32	MUC40	NH2-PPAHGV{Neu5Aca2-6GalNAc}TSAPDTRPAPGS{Neu5Aca2-6GalNAc}TAPPA-OH
	T33	MUC41	NH2-PPAHGVT{Neu5Aca2-6GalNAc}SAPD{Neu5Aca2-6GalNAc}TRPAPGSTAPPA-OH
	T34	MUC42	NH2-PPAHGVT{Neu5Aca2-6GalNAc}SAPDTRPAPG{Neu5Aca2-6GalNAc}STAPPA-OH
	T35	MUC43	NH2-PPAHGVT{Neu5Aca2-6GalNAc}SAPDTRPAPGS{Neu5Aca2-6GalNAc}TAPPA-OH

	T36	MUC44	NH2-PPAHGVTSAPD {Neu5Aca2-6GalNAca} TRPAPG {Neu5Aca2-6GalNAca} STAPPA-OH
	T37	MUC45	NH2-PPAHGVTSAPD {Neu5Aca2-6GalNAca} TRPAPGS {Neu5Aca2-6GalNAca} TAPPA-OH
	T38	MUC46	NH2-PPAHGVTSAPDTRPAPG {Neu5Aca2-6GalNAca} S {Neu5Aca2-6GalNAca} TAPPA-OH
	T39	MUC63	NH2-PPAHGVTSAPDTRPAPGSTAPPA-OH
CA19-9 (SLeA)	T40	SLeA	Neu5Aca2-3Galβ1-3[Fuca1-4]GlcNAc
	T41	SLeA on Lac	Neu5Aca2-3Galβ1-3[Fuca1-4]GlcNAcβ1-3Galβ1-4Glc-
	T42	SLeA on LacNAc	Neu5Aca2-3Galβ1-3[Fuca1-4]GlcNAcβ1-3Galβ1-4GlcNAc-
CA125 (MUC16)	T43	M16-UG	NH2-PVDVGTSGTPSSSPSTTAGP-OH
	T44	M16-T-Tn	NH2-PVDVG(GalNAca)TSGTPSSSPSTTAGP-OH
	T45	M16-S-Tn	NH2-PVDVGT(GalNAca)SGTPSSSPSTTAGP-OH
	T46	M16-T-STn	NH2-PVDVG(Neu5Aca2-6GalNAca)TSGTPSSSPSTTAGP-OH
	T47	M16-S-STn	NH2-PVDVGT(Neu5Aca2-6GalNAca)SGTPSSSPSTTAGP-OH
	T48	M16-Bis-Tn	NH2-PVDVG(GalNAca)T(GalNAca)SGTPSSSPSTTAGP-OH
	T49	M16-Bis-STn	NH2-PVDVG(Neu5Aca2-6GalNAca)T(Neu5Aca2-6GalNAca)SGTPSSSPSTTAGP-OH
Forssman antigen	T50	Forssman antigen pentaose	GalNAca1-3GalNAcβ1-3Galα1-4-Galβ1-4Glc-
Gangliosides	T51	GM1	Neu5Aca2-3(Galβ1-3GalNAcβ1-4)Galβ1-4Glc-
	T52	FucGM1	Fuca1-2Galβ1-3GalNAcβ1-4(Neu5Aca2-3)Galβ1-4Glc-
	T53	GM2	Neu5Aca2-3(GalNAcβ1-4)Galβ1-4Glc-
	T54	GM3	Neu5Aca2-3Galβ1-4Glc-
	T55	GD1a	Neu5Aca2-3Galβ1-3GalNAcb1-4(Neu5Aca2-3)Galβ1-4Glc-
	T56	GD1b	Neu5Aca2-8Neu5Aca2-3(Galβ1-3GalNAcβ1-4)Galβ1-4Glc-
	T57	GD2	Neu5Aca2-8Neu5Aca2-3(GalNAcβ1-4)Galβ1-4Glc-
	T58	GD3	Neu5Aca2-8Neu5Aca2-3Galβ1-4Glc-
	T59	Globo-H	Fuca1-2Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glc-
LacdiNAc	T60	LacdiNAc	GalNAcb1-4GlcNAcb1-2Mana1-6(GalNAcb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4GlcNAc
Neu5Gc Glycans	T61	GcGM1	Neu5Gca2-3(Galβ1-3GalNAcβ1-4)Galβ1-4Glc-
	T62	GcGM2	Neu5Gca2-3(GalNAcβ1-4)Galβ1-4Glc-
	T63	GcGM3	Neu5Gca2-3Galβ1-4Glc-
	T64	Gc2GD1b	Neu5Gca2-8Neu5Gca2-3(Galβ1-3GalNAcβ1-4)Galβ1-4Glc-
	T65	Gc2GD2	Neu5Gca2-8Neu5Gca2-3(GalNAcβ1-4)Galβ1-4Glc-
	T66	Gc2GD3	Neu5Gca2-8Neu5Gca2-3Galβ1-4Glc-
SSEA	T67	SSEA-1 (LeX)	Galβ1-4(Fuca1-3)GlcNAc-
	T68	SSEA-3 (Gb5)	Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glc-
	T69	SSEA-4 (AcGb5)	Neu5Aca2-3Galb1-3GalNAcb1-3Gala-14Galb1-4Glc-
	T70	SLeX	Neu5Aca2-3Galβ1-4(Fuca1-3)GlcNAcβ-
sTRA	T71	Ac3-T1-T2-LN (sTRA)	Neu5Aca2-3Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAc
	T72	A3-G2-T1-LN (sTRA on N-glycan)	Neu5Aca2-3Galb1-3GlcNAcb1-4Galb1-4GlcNAcb1-2Mana1-6(Neu5Aca2-3Galb1-3GlcNAcb1-4Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4GlcNAc
	T73	sTRA on Lac	Neu5Aca2-3Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc-
	T74	sTRA on Core 1 O-glycan	Neu5Aca2-3Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-3GalNAc-

	T75	sTRA on Core 2 O-glycan	Neu5Aca2-3Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1-6(Neu5Aca2-3Galβ1-3)GalNAc
	T76	SLeC	Neu5Aca2-3Galβ1-3GlcNAc
Tri-antennary N-glycan with β6-GlcNAc branch	T77	G(6)30	(GlcNAcβ1-2(GlcNAcβ1-6)Manα1-6)(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
	T78	G(6)3	(Galβ1-4GlcNAcβ1-2(Galβ1-4GlcNAcβ1-6)Manα1-6)(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
	T79	G(6)3S3(3)	(Neu5Aca2-3Galβ1-4GlcNAcβ1-2(Neu5Aca2-3Galβ1-4GlcNAcβ1-6)Manα1-6)(Neu5Aca2-3Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
	T80	G(6)3S3(6)	(Neu5Aca2-6Galβ1-4GlcNAcβ1-2(Neu5Aca2-6Galβ1-4GlcNAcβ1-6)Manα1-6)(Neu5Aca2-6Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
Tn, STn, T, ST antigens	T81	Tn-Ser	GalNAcα-Ser
	T82	STn-Ser	Neu5Aca2-6GalNAcα-Ser
	T83	T-Ser	Galβ1-3GalNAcα-Ser
	T84	ST-Ser	Neu5Aca2-3Galβ1-3GalNAcα-

Controls

NC: Negative control, Print Buffer

PC1: Positive control 1, a biotinylated probe (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)

Array Marker: Anti-Human IgG, Cy3 (0.01 mg/ml) and anti-Human IgG, Alexa555 (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

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 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

Considerations Before Starting the Experiment

1. **Preparation of Buffers:**
 - Ensure that Glycan Array Blocking Buffer (GABB) and Glycan Array Assay Buffer (GAAB) are ready for glycan microarray analysis.
2. **BSA Addition:**
 - If needed, add BSA to GAAB or LAAB to reduce non-specific binding.
 - Prepare a 1% BSA assay buffer by adding BSA to GAAB or LAAB and filter through a 0.2 μm PVDF membrane filter.
3. **Avoiding Dryness:**
 - The array surface is extremely sensitive to dryness. Ensure the array does not dry at any point during the assay.
 - Avoid handling multiple subarrays simultaneously to prevent drying out.
4. **Array Formats and Volumes:**
 - Common array formats: 8, 16, or 24 subarrays.
 - For 8-subarray format: Use 200 μL per subarray.
 - For 16-subarray format: Use 100 μL per subarray.
 - For 24-subarray format: Use 50-80 μL per subarray.
 - Minimal volumes: 60 μL per well for 16-subarray cassettes and 80 μL for 8-subarray cassettes.
 - Caution: Using minimal volumes increases the risk of drying out and may cause signal variation. Ensure samples are homogeneous and thoroughly mixed.
5. **Sample Preparation:**
 - Dilute glycan-binding protein samples or secondary antibodies in Glycan Array Assay Buffer.
 - Recommended concentration range for protein samples: 50 $\mu\text{g}/\text{mL}$ to 0.1 $\mu\text{g}/\text{mL}$. Experiment to find the optimal concentration for highest binding signals with the lowest background.
 - For fluorescently labeled streptavidin, use a concentration of 0.2 $\mu\text{g}/\text{mL}$.
6. **Storage of Microarray Slides and Buffers:**
 - Store microarray slides and buffers at 4°C if assayed within 24 hours of receipt.
 - For long-term storage, keep microarray slides at -20°C. Avoid multiple freeze-thaw cycles.
 - Use slides and buffers within 12 months. Allow slides to equilibrate to room temperature for at least 20 minutes before opening.
 - After opening, reseal unused slides in a moisture barrier bag with a desiccant and refreeze. Handle slides in a dust-free environment, wearing gloves and holding slides by the edges.
 - When adding samples, avoid touching the pipette tip to the array surface. When removing samples, gently touch the pipette tip to the corner of the well and tip the slide.

Analyzing Biological Samples with Glycan Microarray

Part 1: Blocking

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

1. Let the microarray slide equilibrate to room temperature (20-30 minutes) before opening the moisture barrier bag.
2. Assemble the slide into a hybridization chamber device.
3. Add the Glycan Array Blocking Buffer (GABB) to each subarray well of the assembled hybridization chamber device:
 - 100 μL for each subarray of a 16-subarray chamber device
 - 200 μL for each subarray of an 8-subarray chamber device
4. Cover the hybridization chamber device with adhesive film to prevent evaporation and incubate the slide on a shaker at 80 rpm for 30 minutes. Ensure the orbital shaker is completely flat to avoid variations in binding and detection. Longer incubation times are acceptable but not necessary.
5. After 30 minutes, add 100 μL of Glycan Array Assay Buffer (GAAB) to each subarray well of a 16-subarray chamber device (or 200 μL of GAAB to each subarray well of an 8-subarray chamber device). Aspirate the liquid out from each well, ensuring that some liquid remains to cover the surface.
6. Add another 100 μL of GAAB to each subarray well of a 16-subarray chamber device (or 200 μL of GAAB to each subarray well of an 8-subarray chamber device). Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
7. After incubation, aspirate all the liquid out from each well. Then add 100 μL of GAAB to each subarray well of a 16-subarray chamber device (or 200 μL of GAAB to each subarray well of an 8-subarray chamber device). Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
8. After incubation, aspirate all the liquid out from each well. Then add 100 μL of GAAB to each subarray well of a 16-subarray chamber device (or 200 μL of GAAB to each subarray well of an 8-subarray chamber device). Incubate at room temperature for 5 minutes at 80 rpm on a shaker.

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 the blocking buffer from each well by gently touching a pipette tip to the corner of the well. We recommend doing this one subarray at a time, not handling multiple subarrays simultaneously to avoid drying out the microarray slide surface.
3. Immediately apply the glycan-binding protein sample of interest to each well.
4. Seal the wells with adhesive film to prevent evaporation. If the sample is fluorescently labeled, cover it with aluminum foil to keep it in the dark.
5. Incubate on the shaker for 1 hour at 80 rpm. Longer incubation times may increase the 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.
6. If your glycan-binding protein samples are fluorescently labeled, go directly to Part 6 – Final Wash and Dry.

Part 3: Wash

We recommend doing the following procedure using a multi-channel pipette.

1. **Initial Wash:**
 - After incubating the samples for 1 hour at room temperature, use a multi-channel pipette to add 100 μL of Glycan Array Assay Buffer (GAAB) to each subarray well of a 16-subarray chamber device (or 200 μL of GAAB to each subarray well of an 8-subarray chamber device).
 - Aspirate the liquid out using a multi-channel aspirating needle device from each well, ensuring not all the liquid is aspirated out, leaving enough liquid to cover the surface.

- Repeat the addition and aspiration of GAAB three times. For each repetition, add 100 μL (or 200 μL for an 8-subarray device) of GAAB, aspirate, and ensure some liquid remains to cover the surface. Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
- 2. **Subsequent Washes:**
 - After the initial wash and incubation, aspirate all the liquid out from each well using a multi-channel aspirating needle device.
 - Add 100 μL of GAAB to each subarray well of a 16-subarray chamber device (or 200 μL of GAAB to each subarray well of an 8-subarray chamber device).
 - Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
- 3. **Final Wash:**
 - After the second incubation, aspirate all the liquid out from each well using a multi-channel aspirating needle device.
 - Add 100 μL of GAAB to each subarray well of a 16-subarray chamber device (or 200 μL of GAAB to each subarray well of an 8-subarray chamber device).
 - Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
- 4. **Proceed to the Next Step:**
 - If your glycan-binding sample is biotinylated, go directly to Part 5 – Fluorescent Staining.

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

1. **Adding Secondary Antibody:**
 - After removing all the GAAB using a multi-channel aspirating needle device, immediately add the secondary biotinylated antibody to each well using a multi-channel pipette.
2. **Incubation:**
 - Seal the wells with adhesive film and incubate on the shaker for 1 hour at 80 rpm. Longer incubation times are acceptable but not necessary.

Part 5: Fluorescent Staining

1. **Adding Streptavidin:**
 - After completely removing the GAAB using a multi-channel aspirating needle device, immediately add the fluorescently labeled streptavidin sample using a multi-channel pipette.
2. **Incubation:**
 - 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 times are acceptable but not necessary.

Part 6: Final Wash and Dry

1. **Initial Wash:**
 - After incubating the secondary antibody or streptavidin for 1 hour at room temperature, use a multi-channel pipette to add 100 μL of GAAB to each subarray well of a 16-subarray chamber device (or 200 μL of GAAB to each subarray well of an 8-subarray chamber device).
 - Aspirate the liquid out using a multi-channel aspirating needle device from each well, ensuring not all the liquid is aspirated out, leaving enough liquid to cover the surface.
 - Repeat the addition and aspiration of GAAB three times. For each repetition, add 100 μL (or 200 μL for an 8-subarray device) of GAAB, aspirate, and ensure some liquid remains to cover the surface. Incubate at room temperature for 5 minutes at 80 rpm on a shaker.

2. **Subsequent Washes:**
 - After the initial wash and incubation, aspirate all the liquid out from each well using a multi-channel aspirating needle device.
 - Add 100 μL of GAAB to each subarray well of a 16-subarray chamber device (or 200 μL of GAAB to each subarray well of an 8-subarray chamber device).
 - Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
3. **Final Wash:**
 - After the second incubation, aspirate all the liquid out from each well using a multi-channel aspirating needle device.
 - Add 100 μL of GAAB to each subarray well of a 16-subarray chamber device (or 200 μL of GAAB to each subarray well of an 8-subarray chamber device).
 - Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
4. **Disassembling the Hybridization Chamber:**
 - Disassemble the hybridization chamber device 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. **Immersing the Slide:**
 - Immediately immerse the slide in a Coplin jar 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. Place the jar or beaker on a shaker at 80 rpm for 10 minutes.
6. **Rinsing with Water:**
 - Decant the buffer from the jar while holding the slide in place (only touch the edge of the slide) and then add sterile de-ionized water to immerse the slide. Place the jar on the shaker at 80 rpm for 5 minutes.
7. **Repeat Rinsing:**
 - Decant the water from the jar. Repeat once more with fresh de-ionized water.
8. **Drying the Slide:**
 - Allow the slide to dry by using a microarray slide centrifuge completely in a clean, dust-free environment before scanning.

Part 7: Data Acquisition and Analysis

1. **Scanning the Slide:**
 - 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 being saturated (saturated positive control signal is okay).
2. **Analyzing Data:**
 - Analyze data with microarray analysis software. If there is specific binding, the signal intensity should be higher than the background signal (the area where there are no printed spots). The fluorescent signal due to specific binding to your sample of interest should be dose-dependent with your sample dilution (unless the sample concentration range is too high and glycan-binding is saturated) and should have a positive binding signal after the signal from control assays has been subtracted.
3. **Quantifying Signal Intensities:**
 - Our standard method of comparing signal intensities is to quantify the median signal intensity data and subtract the background intensity. Subtracting signals 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.
4. **Interpreting 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 a fluorescence signal regardless of the assay. It is there primarily to aid with the orientation of the array map during analysis.
- **Biotinylated Mannose (PC1):** This positive control will bind directly to the fluorescent-labeled streptavidin. If your glycan-binding protein sample is already fluorescently labeled, or in any case where the addition of fluorescently labeled 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 the blood that is a primary component of humoral immunity. If the glycan-binding or secondary antibody sample is an anti-IgG from a human, rabbit, or mouse, it should bind to the respective IgG control.

Typical Binding Assay Result from the Tumor Associated Glycan Microarray

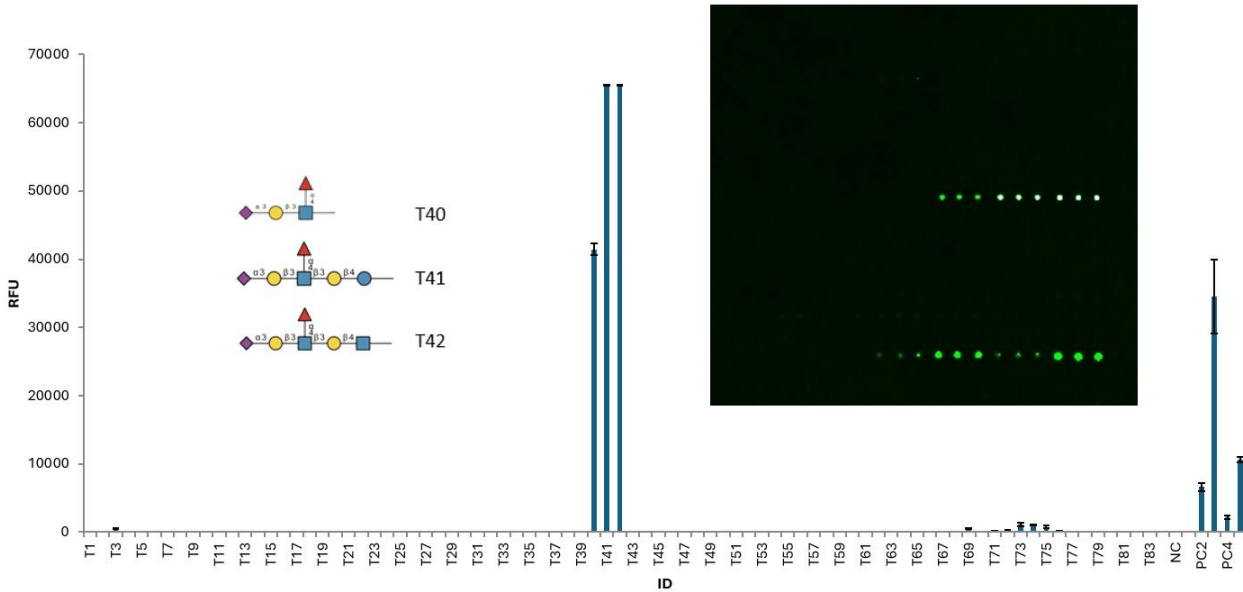
To demonstrate the performance of the Tumor-Associated Glycan (TAG) Array, a panel of well-characterized antibodies targeting clinically relevant tumor-associated carbohydrate antigens was tested across the 84-glycan panel. The array was incubated with each antibody at indicated concentrations followed by fluorescent secondary detection, and the slides were scanned using high laser power to capture the binding signals.

MUC1 Antibody Profiling

The anti-MUC1 antibody displayed strong and selective binding to the MUC1 glycopeptide panel (T9-T39). At 1 µg/mL, high fluorescence signals were observed across most MUC1 peptide variants, including peptides carrying single and multiple GalNAc (Tn antigen) or sialyl-Tn (Neu5Acα2-6GalNAc) modifications. Peak signals exceeded several thousand RFU for multiple glycopeptide variants, indicating strong recognition of the glycosylated MUC1 tandem repeat region. Importantly, binding signals for non-mucin glycans on the array remained near background, confirming that the antibody specifically recognizes the MUC1 glycopeptide scaffold rather than isolated glycan motifs.

Array Scan Image

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



Quantitative Data

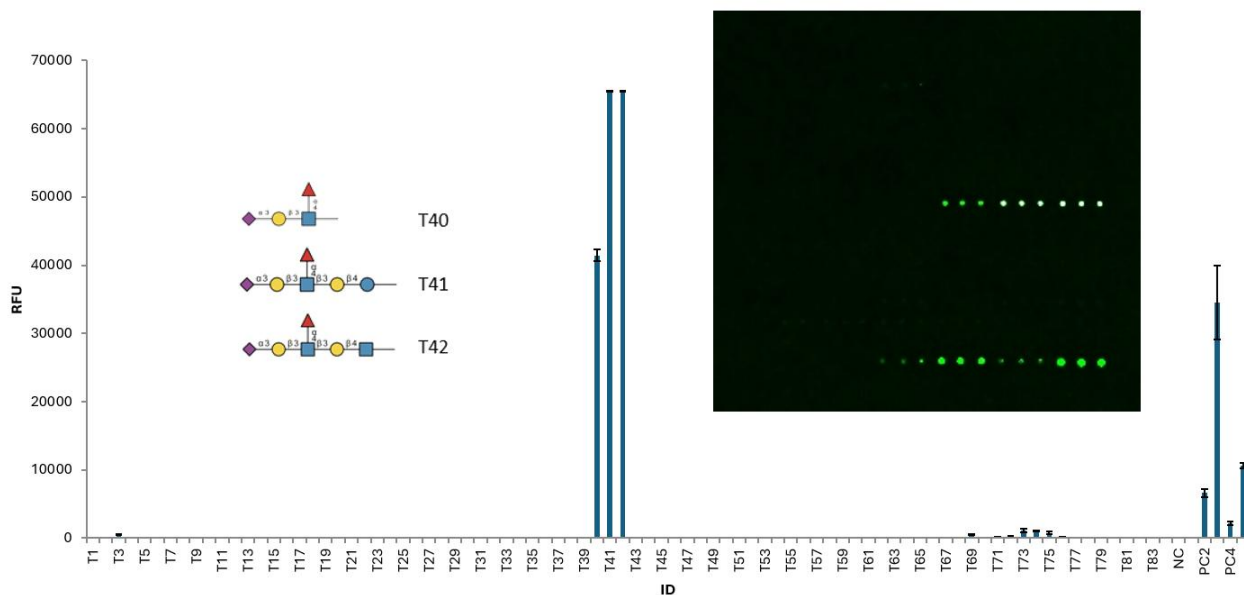
Data was generated by analyzing scanned microarray images.

CA19-9 Antibody Specificity

The CA19-9 antibody showed strong binding to glycans containing the sialyl-Lewis A (SLeA) epitope. At 5 $\mu\text{g/mL}$, the highest signals were observed for glycans T40–T42, which represent SLeA structures presented in different glycan contexts. These signals were significantly higher than background signals observed for unrelated glycans across the array. Additional moderate signals were detected on related glycans that contain similar terminal motifs, but the overall binding pattern clearly clustered around the SLeA-containing structures.

Array Scan Image

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



Quantitative Data

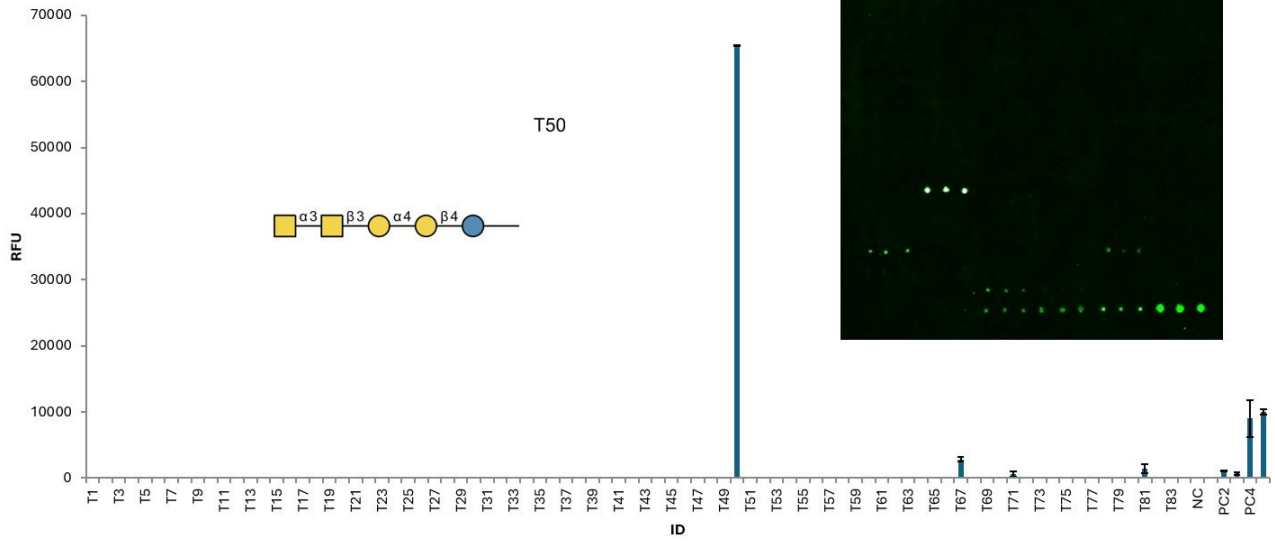
Data was generated by analyzing scanned microarray images.

Forssman antigen antibody

The Forssman antigen is a pentasaccharide glycolipid, which is normally absent from most human tissues but can be aberrantly expressed in certain cancers and tumor-derived cell lines. When the anti-Forssman antibody (10 $\mu\text{g/mL}$) was applied to the TAG array, a strong and highly selective binding signal was observed at the Forssman antigen spot (T50). In contrast, most other glycans on the array, including structurally related glycosphingolipids such as GM1, GM2, GM3, GD2, and GD3, produced only background-level signals. This selective binding pattern demonstrates that the TAG Array can clearly distinguish the Forssman antigen from other closely related glycan structures.

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	<ul style="list-style-type: none"> • Concentration of sample of interest 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. 	<ul style="list-style-type: none"> • Use a lower concentration range of samples. Consider a wider range if you are unsure where the detection limit is. Use control assays to determine which sample is causing high background. • Apply longer times for washing steps and use a higher shaking rate • Make sure wash buffer and sample is completely removed before the next step • Make sure adhesive film fully seals the wells to avoid evaporation • Centrifuge the samples prior to assay to avoid adding irrelevant particles. Make sure buffers are filtered. • If you think that the protein is aggregating during incubation, try shaking at a higher speed
Signal Variation	<ul style="list-style-type: none"> • Slide drying out during assay • Binding samples are not equally distributed in the wells • Glycan-binding protein aggregation during incubation • Bubbles during incubation 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • Cross contamination between wells or other sources • Sample contamination 	<ul style="list-style-type: none"> • 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