CatchAll Glycan Array User Manual

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Introduction

Glycans are carbohydrate-based polymers usually found on proteins and lipids on the eukaryotic cell surface. They help maintain normal cellular homeostasis and play essential roles in cell growth and survival. For example, glycans attached to the cell surface provide physical protective barriers, whereas those found in the intracellular matrix facilitate protein folding, sorting, and secretion. Glycans are also crucial for cell communication, acting as ligands for cell-to-cell interactions. For example, glycans are ligands of many immune-modulating receptors, and specific ligations dictate either enhancing or suppressive immune responses. Glycans also provide scaffolds for invading pathogens to enter the cell for infection. Glycans can be found on the surface of bacteria, viruses, fungi, and other microorganisms. Molecular mimicry of host glycans helps pathogens evade immune surveillance.

The properties and functions of the glycans are highly dependent on the monosaccharide unit, their combination, bonding position, and the type of branching. An enormous diversity of glycan structures exist, and many of these structures still need to be characterized. As a result, there has been an increasing interest in understanding the structural and functional basis of these complex carbohydrate molecules.

ZBiotech has developed a robust microarray platform that allows researchers to exploit the interactions between 116 structurally – defined glycans and biological samples, such as proteins, antibodies, cells, cell lysate, serum, vesicles, bacteria, or viral particles. These glycans represent a diverse collection of carbohydrate structures. These structures range from mammalian oligosaccharides, N-glycans, O-glycans, glycosphingolipid glycans, blood group antigen glycans, glycosaminoglycans, sialylated glycans to human milk oligosaccharides (HMOs). Each array contains 8 or 16 identical subarrays, enabling the simultaneous analysis of multiple samples. The CatchAll glycan array provides high-throughput and reliable glycan-binding information with a simple assay format that only requires a small sample volume. Results can be obtained in only a few hours, making investigating glycan binding easy and efficient. The CatchAll array can be customized to meet individual client needs. Assay services are available upon request.

This manual is a comprehensive guide to help the researcher acquire clear results from the assay. Please read through carefully before starting your experiment.

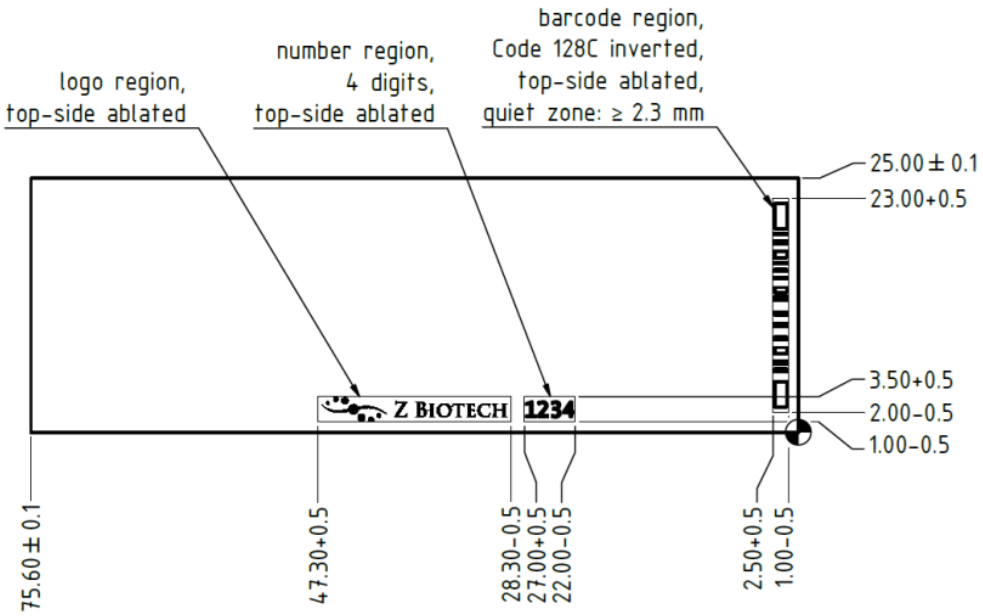
Handling and Storage

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 a 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.

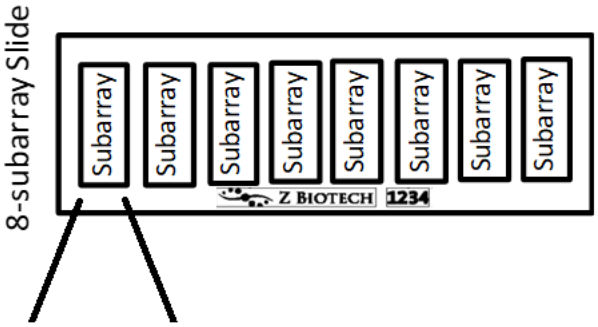
Allow the bag of slides to equilibrate to room temperature at least 20 minutes before opening. After opening, re-seal any unused slides in the moisture barrier bag with a desiccant inside and refreeze. Handle the slides in a dust-free environment. Wear gloves and hold the slides on the edges. When adding samples, do not touch the pipette tip to the array surface. When removing the 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

CatchAll Glycan Array slides have 8 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



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166	166	166	166	NC1	NC1	NC1	NC1	NC2	NC2	NC2	NC2	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	M	M	M	M

CatchAll Glycan Identification List:

Glycan Type	Subtype	ID	Name	Structure
Monosaccharide (6)		CA1	D-Glucose	Glc
		CA2	D-GlcNAc	GlcNAc
		CA3	D-Galactose	Gal
		CA4	D-GalNAc	GalNAc
		CA5	D-Mannose	Man
		CA6	L-Fucose	Fuc
Blood Group and Lewis Antigens (15)		CA7	Lewis A trisaccharide	Galβ1-3(Fucα1-4)GlcNAc
		CA8	Lewis B tetrasaccharide	Fucα1-2Galβ1-3(Fucα1-4)GlcNAc
		CA9	Lewis X trisaccharide	Galβ1-4(Fucα1-3)GlcNAc
		CA10	Lewis Y pentasaccharide	Fucα1-2Galβ1-4(Fucα1-3)GlcNAcβ1-3Gal
		CA11	3'-Sialyl Lewis A	Neu5Acα2-3Galβ1-3(Fucα1-4)GlcNAc

		CA12	3'-Sialyl Lewis X	Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAc
		CA13	3'-Sulfated Lewis A	(3S)Galβ1-3(Fucα1-4)GlcNAc
		CA14	3'-Sulfated Lewis X	(3S)Galβ1-4(Fucα1-3)GlcNAc
		CA15	Blood Group A pentasaccharide type I	GalNAcα1-3(Fucα1-2)Galβ1-3GlcNAcβ1-3Gal
		CA16	Blood group A pentasaccharide type II	GalNAcα1-3(Fucα1-2)Galβ1-4GlcNAcβ1-3Gal
		CA17	Blood Group A tetrasaccharide type VI	GalNAcα1-3(Fucα1-2)Galβ1-4Glc
		CA18	Blood group B hexasaccharide type I	Galα1-3-(Fucα1-2)Galβ1-3GlcNAcβ1-3Galβ1-4Glc
		CA19	Blood group B hexasaccharide type II	Galα1-3-(Fucα1-2)Galβ1-4GlcNAcβ1-3Galβ1-4Glc
		CA20	Blood Group H type I trisaccharide	Fucα1-2Galβ1-3GlcNAc
		CA21	Blood Group H type II trisaccharide	Fucα1-2Galβ1-4GlcNAc
GSL Glycans (19)		CA22	Gangliotetraose	Galβ1-3GalNAcβ1-4Galβ1-4Glc
		CA23	GM1	Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glc
		CA24	Fucosyl GM1	Fucα1-2Galβ1-3GalNAcβ1-4(Neu5Acα2-3)Galβ1-4Glc

		CA25	GM2	Neu5Acα2-3(GalNAcβ1-4)Galβ1-4Glc
		CA26	GD1a	Neu5Acα2–3Galβ1–3GalNAcβ1–4(Neu5Acα2–3)Galβ1–4Glc
		CA27	GD1b	Neu5Acα2–8Neu5Acα2–3(Galb1–3GalNAcb1–4)Galβ1–4Glc
		CA28	GD2	Neu5Acα2–8Neu5Acα2–3(GalNAcb1–4)Galβ1–4Glc
		CA29	GD3	Neu5Acα2–8Neu5Acα2–3Galβ1–4Glc
		CA30	GT1a	Neu5Acα2–8Neu5Acα2–3Galβ1–3GalNAcβ1–4(Neu5Acα2–3)Galβ1–4Glc
		CA31	GT1c	Galβ1–3GalNAcβ1–4(Neu5Acα2–8Neu5Acα2–8Neu5Acα2–3)Galβ1–4Glc
		CA32	GT2	GalNAcβ1-4(Neu5Acα2–8Neu5Acα2–8Neu5Acα2–3)Galβ1–4Glc
		CA33	GT3	Neu5Acα2–8Neu5Acα2–8Neu5Acα2–3Galβ1–4Glc
		CA34	Forssman antigen pentaose	GalNAcα1-3GalNAcβ1-3Galα1-4-Galβ1-4Glc
		CA35	Globo-H hexaose	Fucα1-2Galβ1-3GalNAcβ1-3Galα1-4Galβ1-4Glc
		CA36	Globotriose, Gb3	Galα1–4Galβ1–4Glc
		CA37	Globo-N-tetraose, Gb4	GalNAcβ1–3Galα1–4Galβ1–4Glc

		CA38	Globoisotetraose	GalNAcβ1–3Galα1–3Galβ1–4Glc
		CA39	Globopentaose (SSEA-3)	Galβ1–3GalNAcβ1–3Galα1–4Galβ1–4Glc
		CA40	SSEA-4 hexaose	Neu5Acα2–3Galβ1–3GalNAcβ1–3Galα1–4Galβ1–4Glc
HMO (15)		CA41	Lactose	Galβ1-4Glc
		CA42	2'-Fucosyllactose	Fucα1-2Galβ1-4Glc
		CA43	3-Fucosyllactose	Galβ1-4(Fucα1-3)Glc
		CA44	3'-Sialyllactose	Neu5Acα2–3Galβ1–4Glc
		CA45	6'-Sialyllactose	Neu5Acα2-6Galβ1-4Glc
		CA46	3'-a-Sialyl-N-acetyllactosamine	Neu5Acα2-3Galβ1-4GlcNAc
		CA47	6'-a-Sialyl-N-acetyllactosamine	Neu5Acα2-6Galβ1-4GlcNAc
		CA48	Lacto-N-tetraose, LNT	Galβ1-3GlcNAcβ1-3Galβ1-4Glc
		CA49	Lacto-N-neotetraose, LNnT	Galβ1-4GlcNAcβ1-3Galβ1-4Glc
		CA50	Lacto-N-fucopentaose I	Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc

		CA51	Lacto-N-fucopentaose II	Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glc
		CA52	Lacto-N-fucopentaose III	Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc
		CA53	LS-tetrasaccharide a (LsTa)	Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc
		CA54	LS-tetrasaccharide c (LsTc)	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4Glc
		CA55	Disialyllacto-N-tetraose (DSLNT)	Neu5Acα2-3Galβ1-3(Neu5Acα2-6)GlcNAcβ1-3Galβ1-4Glc
Glucan (10)		CA56	Sophorose	Glcβ1-2Glc
		CA57	Gentiobiose	Glcβ1-6Glc
		CA58	Nigerose	Glcα1-3Glc
		CA59	Kojibiose	Glcα1-2Glc
		CA60	Laminaritriose	Glcβ1-3Glcβ1-3Glc
		CA61	Maltotetraose	Glcα1-4Glcα1-4Glcα1-4Glc
		CA62	Cellotetraose	Glcβ1-4Glcβ1-4Glcβ1-4Glc
		CA63	1,3:1,4 β-Glucotetraose	Glcβ1-4Glcβ1-3Glcβ1-4-Glc

		CA64	6-a-D-Glucopyranosyl maltotriose	Glcα1-6Glcα1-4Glcα1-4Glc
		CA65	Isomaltotetraose	Glcα1-6Glcα1-6Glcα1-6Glc
General Oligosaccharides (53)		CA66	2-O-a-D-Glucopyranosyl-D-galactopyranose	Glcα1-2Gal
		CA67	2-O-(a-D-Galactopyranosyl)-D-galactopyranose	Galα1-2Gal
		CA68	2-O-(b-D-Galactopyranosyl)-D-galactopyranose	Galβ1-2Gal
		CA69	Lactose 3'-sulfate	(3S)Galβ1-4Glc
		CA70	3-O-(a-D-Galactopyranosyl)-D-galactopyranose	Galα1-3Gal
		CA71	3-O-(a-D-Galactopyranosyl)-D-glucopyranose	Galα1-3Glc
		CA72	3-O-(b-D-Galactopyranosyl)-D-galactopyranose	Galβ1-3Gal
		CA73	3-O-(b-D-Galactopyranosyl)-D-glucopyranose	Galβ1-3Glc
		CA74	4-O-(a-D-Galactopyranosyl)-D-galactopyranose	Galα1-4Gal
		CA75	4-O-(a-D-Galactopyranosyl)-D-glucopyranose	Galα1-4Glc
		CA76	4-O-(b-D-Galactopyranosyl)-D-galactopyranose	Galβ1-4Gal

		CA77	4-O-(b-D-Galactopyranosyl)-D-mannopyranose	Galβ1-4Man
		CA78	6-O-(a-D-Galactopyranosyl)-D-galactopyranose	Galα1-6Gal
		CA79	6-O-(b-D-Galactopyranosyl)-D-galactopyranose	Galβ1-6Gal
		CA80	6-O-(b-D-Galactopyranosyl)-D-glucopyranose	Galα1-6Glc
		CA81	3-O-(b-D-Galactopyranosyl)-D-arabinose	Galβ1-3Ara
		CA82	Rutinose	Rhaα1-6Glc
		CA83	2-Acetamido-2-deoxy-3-O-(a-D-galactopyranosyl)-D-galactopyranose	Galα1-3GalNAc
		CA84	2-Acetamido-2-deoxy-3-O-(a-L-fucopyranosyl)-D-glucopyranose	Fucα1-3GlcNAc
		CA85	2-Acetamido-2-deoxy-4-O-(a-D-galactopyranosyl)-D-glucopyranose	Galα1-4GlcNAc
		CA86	N-Acetyl-D-lactosamine	Galβ1-4GlcNAc
		CA87	2-Acetamido-2-deoxy-4-O-(a-L-fucopyranosyl)-D-glucopyranose	Fucα1-4GlcNAc
		CA88	2-Acetamido-2-deoxy-4-O-(b-D-galactopyranosyl)-6-sulfo-b-D-glucopyranose	Galβ1-4(6S)GlcNAc
		CA89	2-Acetamido-2-deoxy-6-O-(a-L-	Fucα1-6GlcNAc

			fucopyranosyl)-D-glucopyranose	
		CA90	2-Acetamido-2-deoxy-6-O-(b-D-galactopyranosyl)-D-galactopyranose	Galβ1-6GalNAc
		CA91	2-Acetamido-3-O-(2-acetamido-2-deoxy-a-D-galactopyranosyl)-2-deoxy-D-galactopyranose	GalNAcα1-3GalNAc
		CA92	2-O-(2-Acetamido-2-deoxy-b-D-glucopyranosyl)-D-mannopyranose	GlcNAcβ1-2Man
		CA93	3-O-(2-Acetamido-2-deoxy-a-D-galactopyranosyl)-D-galactopyranose	GalNAcα1-3Gal
		CA94	3-O-(2-Acetamido-2-deoxy-b-D-galactopyranosyl)-D-galactopyranose	GalNAcβ1-3Gal
		CA95	3-O-(2-Acetamido-2-deoxy-b-D-glucopyranosyl)-D-mannopyranose	GlcNAcβ1-3Man
		CA96	6’-Sulfated-N-acetyllactosamine	(6S)Galβ1-4GlcNAc
		CA97	Galacto-N-biose	Galβ1-3GalNAc
		CA98	3'-Sialylgalactose (GM4)	Neu5Acα2-3Galβ
		CA99	6'-Sialylgalactose	Neu5Acα2-6Gal
		CA100	3'-Sialylgalacto-N-biose	Neu5Acα2-3Galβ1-3GalNAc

		CA101	3'-Galactosyllactose	Galβ1-3Galβ1-4Glc
		CA102	4'-Galactosyllactose	Galβ1-4Galβ1-4Glc
		CA103	6'-Galactosyllactose	Galβ1-6Galβ1-4Glc
		CA104	3a,4b-Galactotriose	Galα1-3Galβ1-4Gal
		CA105	Gala1-3Galb1-4Glc	Galα1-3Galβ1-4Glc
		CA106	4-O-(6-O-[2-Acetamido-2-deoxy-b-D-glucopyranosyl]-b-D-galactopyranosyl)-D-glucopyranose	GlcNAcβ1-6Galβ1-4Glc
		CA107	4-O-[3-O-(2-Acetamido-2-deoxy-a-D-galactopyranosyl)-b-D-galactopyranosyl]-D-glucose	GalNAcα1-3Galβ1-4Glc
		CA108	1,2-a-1,2-a-L-Rhamnotriose	Rhaα1-2Rhaα1-2Rha
		CA109	4'-O-(2-Acetamido-2-deoxy-3-O-(b-D-galactopyranosyl)-b-D-galactopyanosyl)-b-D-lactose	Galβ1-3GalNAcβ1-4Galβ1-4Glc
		CA110	2-O-(a-D-Mannopyranosyl)-D-mannopyranose	Manα1-2Man
		CA111	3-O-(a-D-Mannopyranosyl)-D-mannopyranose	Manα1-3Man

		CA112	4-O-(a-D-Mannopyranosyl)-D-mannose	Manα1-4Man
		CA113	6-O-(a-D-Mannopyranosyl)-D-mannose	Manα1-6Man
		CA114	a-D-Galactosyl-mannotriose	Manβ1-4Manβ1-4(Galα1-6)Man
		CA115	a1-3[a1-6]a1-6[a1-3]Mannopentaose	Manα1-6(Manα1-3)Manα1-6(Manα1-3)Man
		CA116	1,4-b-D-Mannotetraose	Manβ1-4Manβ1-4Manβ1-4Man
		CA117	1,4-b-D-Xylotetraose	Xylβ1-4Xylβ1-4Xylβ1-4Xyl
		CA118	Xyloctaose	
N-glycan (28)	**Complex**	CA119		Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
		CA120		Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
		CA121		Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-6(Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
		CA122		Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-6(Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
	Hybrid	CA123		Manα1-6(Manα1-3)Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
		CA124		Manα1-6(Manα1-3)Manα1-6(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-

		CA125		Manα1-6(Manα1-3)Manα1-6(Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
		CA126		Manα1-6(Manα1-3)Manα1-6(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
		CA127		Manα1-6(Manα1-3)Manα1-6(Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
		CA128		Manα1-6(Manα1-3)Manα1-6(Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
	High-mannose	CA129	NG-016	Manα1-6(Manα1-3)Manα1-6(Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
		CA130	NG-004	Manα1-6(Manα1-3)Manα1-6(Manα1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
		CA131	NG-003	Manα1-6(Manα1-3)Manα1-6(Manα1-2Manα1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
		CA132	NG-001	Manα1-2Manα1-6(Manα1-3)Manα1-6(Manα1-2Manα1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
		CA133	Man-9	Manα1-2Manα1-6(Manα1-2Manα1-3)Manα1-6(Manα1-2Manα1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
	Bisecting	CA134		GlcNAcβ1-2Manα1-6(GlcNAcβ1-4)(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
		CA135		Galβ1-4GlcNAcβ1-2Manα1-6(GlcNAcβ1-4)(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
		CA136		Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-6(GlcNAcβ1-4)(Neu5Acα2-3Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
		CA137		Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6(GlcNAcβ1-4)(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-

		CA138		Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-6(GlcNAcβ1-4)(Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
		CA139		Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-6(GlcNAcβ1-4)(Neu5Acα2-3Galβ1-4(Fucα1-3)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-
	Core-fucose	CA140		GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4(Fucα1-6)GlcNAc-
		CA141		Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4(Fucα1-6)GlcNAc-
		CA142		Galβ1-4GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4(Fucα1-6)GlcNAc-
	LacNAc-extended	CA143	TE024	GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-6(GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
		CA144	TE025	Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-6(Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
		CA145	TE019	Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Acα2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc
	Glycopeptide	CA146		Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-6(Neu5Acα2-6Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc-KVA**N**KT
O-glycan (14)	**Glycoamino acid**	CA147	Tn antigen	GalNAcα-Thr
		CA148	STn antigen	Neu5Acα2-6GalNAcα-Thr
		CA149	Core 1 (T antigen)	Galβ1-3GalNAcα-Thr
		CA150	ST antigen	Neu5Acα2-3Galβ1-3GalNAcα-Ser

		CA151	Core 2	GlcNAcβ1-6(Galβ1-3)GalNAcα-Thr
		CA152	Core 3	GlcNAcβ1-3GalNAcα-Thr
		CA153	Core 4	GlcNAcβ1-6(GlcNAcβ1-3)GalNAcα-Thr
		CA154	Core 6	GlcNAcβ1-6GalNAcα-Ser
		CA155	diST	Neu5Acα2-3Galβ1-3(Neu5Acα2-6)GalNAcα-Ser
	Glycopeptide	CA156	MUC1 Glycopeptide	PPAHGVT(GalNAc)SAPDTRPAPGSTAPPA
		CA157	MUC1 Glycopeptide	PPAHGVTS(GalNAc)APDTRPAPGSTAPPA
		CA158	MUC1 Glycopeptide	PPAHGVTSAPDT(GalNAc)RPAPGSTAPPA
		CA159	MUC1 Glycopeptide	PPAHGVTSAPDTRPAPGS(GalNAc)TAPPA
		CA160	MUC1 Glycopeptide	PPAHGVTSAPDTRPAPGST(GalNAc)APPA
GAG	**HA**	CA161	Hyaluronic Acid Polymer (HA93), Mw 93kDa	ΔGlcAβ1,3 $[GlcNAcβ1,4\ GlcAβ1,3]_n$ GlcNAc
	Heparin	CA162	Heparin dp22 (H22)	ΔUA,2S - GlcNS,6S– $[IdoUA,2S – GlcNS,6S]_{10}$
	CS	CA163	Chondroitin Sulphate Oligosaccharide dp20 (CSO20)	ΔUA - $[GalNAc,6S\ or\ 4S - GlcA]_9$ - GalNAc,6S or 4S

	CSD	CA164	Chondroitin Sulphate D Oligosaccharide dp20 (CSDO20)	ΔUA - [GalNAc,6S or 4S – GlcA +/- 2S]$_9$ – GalNAc,6S
	DS	CA165	Dermatan Sulphate dp20 (DS20)	ΔUAβ1,3 - GalNAc,4S – [IdoA – GalNAc,4S]$_9$
	HS	CA166	Heparan Sulphate Oligosaccharide dp20 (Hep III, high sulphation)	ΔUA - GlcNS – [IdoA +/- 2S - GlcNS]$_8$ – IdoA - GlcNAc

Controls

NC: Negative control, Print Buffer

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

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

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

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

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

Materials Required

- CatchAll Array slide
- 8-subarray 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 labeled 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 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 μg/mL to 0.1 μg/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 μg/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.

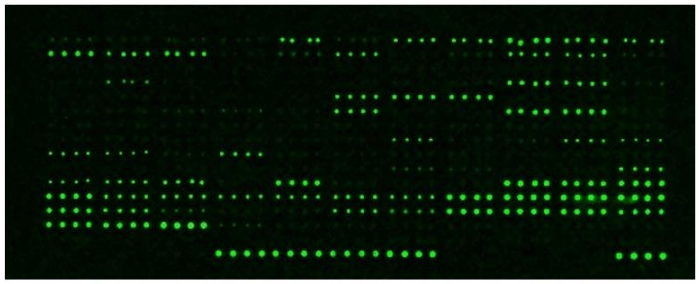
Part 7: Data Acquisition and Analysis

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.

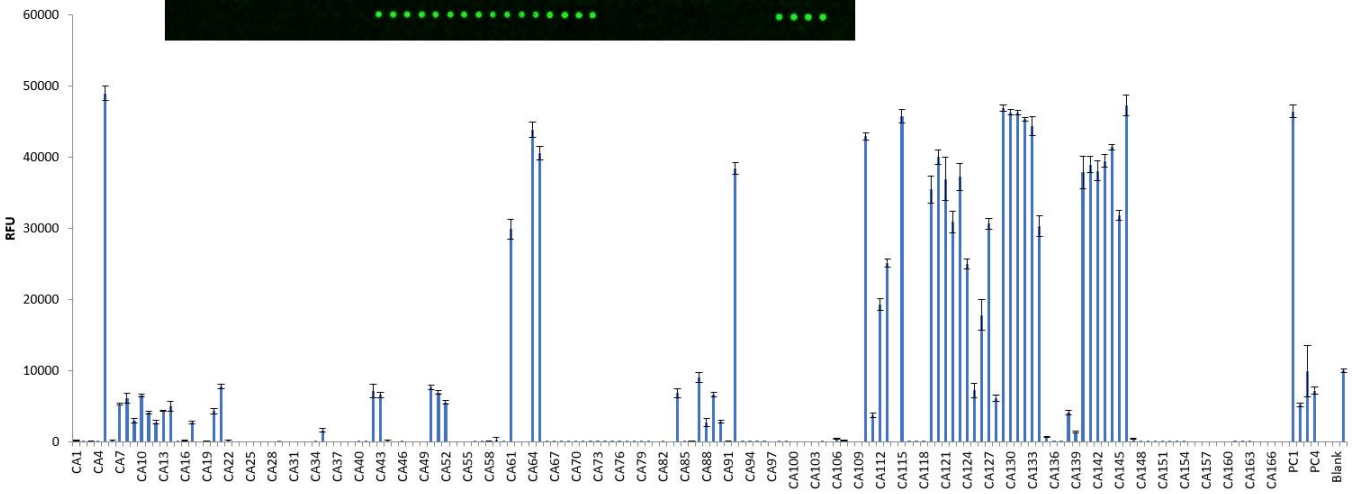
Typical Binding Assay Result from the CatchAll Glycan Array

The CatchAll glycan array was assayed with biotinylated Concanavalin A (Con A) (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. Microarray analysis discloses that ConA mainly binds to high-mannose and complex N-glycans, and glucan glycans, and weakly interacts with Lewis-type 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