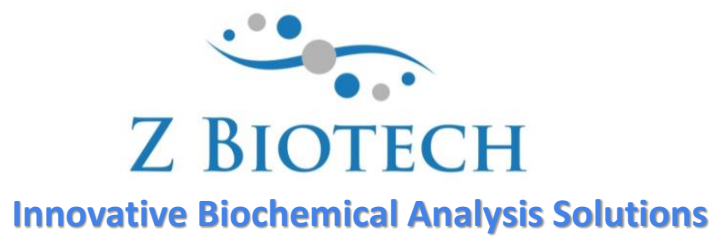


# CatchAll Glycan Array User Manual



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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.

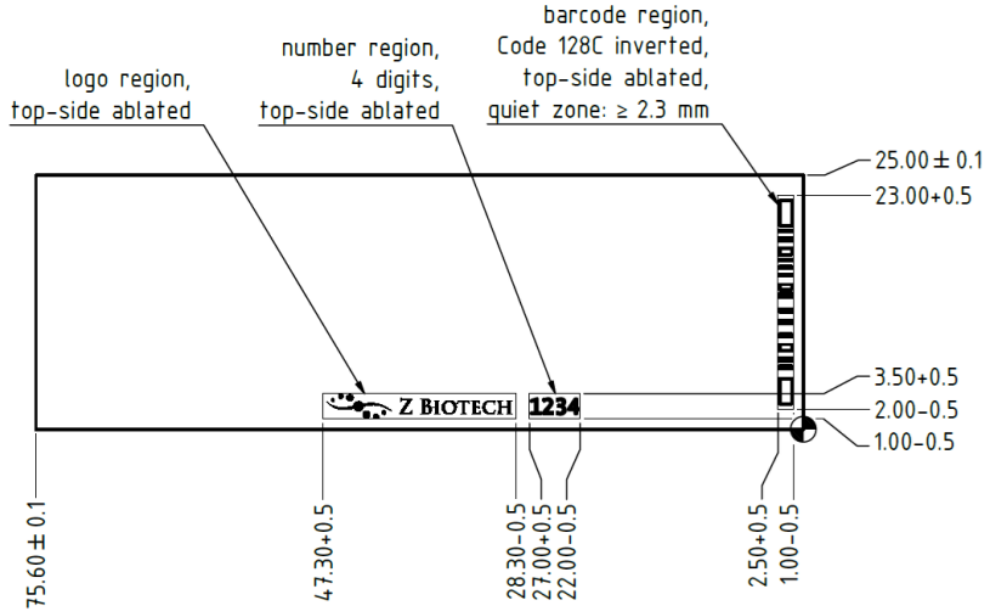
## Handling and Storage

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.

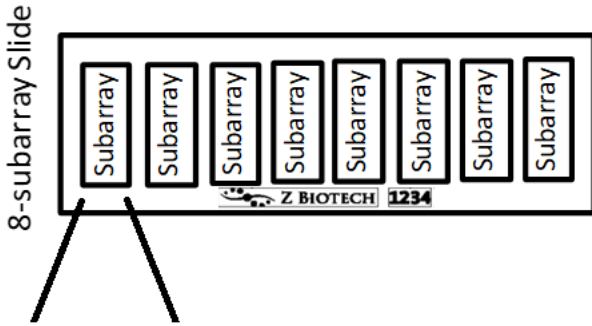
Allow the bag of slides to equilibrate to room temperature at least 20 minutes before opening. After opening, re-seal any unused slides in the moisture barrier bag with a desiccant inside and refreeze. Handle the slides in a dust-free environment. Wear gloves and hold the slides on the edges. When adding 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



1	1	1	1	2	2	2	2	3	3	3	3	4	4	4	4	5	5	5	5	6	6	6	6	7	7	7	7	8	8	8	8	9	9	9	9	10	10	10	10	11	11	11	11
12	12	12	12	13	13	13	13	14	14	14	14	15	15	15	15	16	16	16	16	17	17	17	17	18	18	18	18	19	19	19	19	20	20	20	20	21	21	21	21	22	22	22	22
23	23	23	23	24	24	24	24	25	25	25	25	26	26	26	26	27	27	27	27	28	28	28	28	29	29	29	29	30	30	30	30	31	31	31	31	32	32	32	32	33	33	33	33
34	34	34	34	35	35	35	35	36	36	36	36	37	37	37	37	38	38	38	38	39	39	39	39	40	40	40	40	41	41	41	41	42	42	42	42	43	43	43	43	44	44	44	44
45	45	45	45	46	46	46	46	47	47	47	47	48	48	48	48	49	49	49	49	50	50	50	50	51	51	51	51	52	52	52	52	53	53	53	53	54	54	54	54	55	55	55	55
56	56	56	56	57	57	57	57	58	58	58	58	59	59	59	59	60	60	60	60	61	61	61	61	62	62	62	62	63	63	63	63	64	64	64	64	65	65	65	65	66	66	66	66
67	67	67	67	68	68	68	68	69	69	69	69	70	70	70	70	71	71	71	71	72	72	72	72	73	73	73	73	74	74	74	74	75	75	75	75	76	76	76	76	77	77	77	77
78	78	78	78	79	79	79	79	80	80	80	80	81	81	81	81	82	82	82	82	83	83	83	83	84	84	84	84	85	85	85	85	86	86	86	86	87	87	87	87	88	88	88	88
89	89	89	89	90	90	90	90	91	91	91	91	92	92	92	92	93	93	93	93	94	94	94	94	95	95	95	95	96	96	96	96	97	97	97	97	98	98	98	98	99	99	99	99
100	100	100	100	101	101	101	101	102	102	102	102	103	103	103	103	104	104	104	104	105	105	105	105	106	106	106	106	107	107	107	107	108	108	108	108	109	109	109	109	110	110	110	110
111	111	111	111	112	112	112	112	113	113	113	113	114	114	114	114	115	115	115	115	116	116	116	116	117	117	117	117	118	118	118	118	119	119	119	119	120	120	120	120	121	121	121	121
122	122	122	122	123	123	123	123	124	124	124	124	125	125	125	125	126	126	126	126	127	127	127	127	128	128	128	128	129	129	129	129	130	130	130	130	131	131	131	131	132	132	132	132
133	133	133	133	134	134	134	134	135	135	135	135	136	136	136	136	137	137	137	137	138	138	138	138	139	139	139	139	140	140	140	140	141	141	141	141	142	142	142	142	143	143	143	143
144	144	144	144	145	145	145	145	146	146	146	146	147	147	147	147	148	148	148	148	149	149	149	149	150	150	150	150	151	151	151	151	152	152	152	152	153	153	153	153	154	154	154	154
155	155	155	155	156	156	156	156	157	157	157	157	158	158	158	158	159	159	159	159	160	160	160	160	161	161	161	161	162	162	162	162	163	163	163	163	164	164	164	164	165	165	165	165
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
<b>Monosaccharide (6)</b>		CA1	D-Glucose	Glc
		CA2	D-GlcNAc	GlcNAc
		CA3	D-Galactose	Gal
		CA4	D-GalNAc	GalNAc
		CA5	D-Mannose	Man
		CA6	L-Fucose	Fuc
<b>Blood Group and Lewis Antigens (15)</b>		CA7	Lewis A trisaccharide	Gal $\beta$ 1-3(Fuc $\alpha$ 1-4)GlcNAc
		CA8	Lewis B tetrasaccharide	Fuc $\alpha$ 1-2Gal $\beta$ 1-3(Fuc $\alpha$ 1-4)GlcNAc
		CA9	Lewis X trisaccharide	Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc
		CA10	Lewis Y pentasaccharide	Fuc $\alpha$ 1-2Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc $\beta$ 1-3Gal
		CA11	3'-Sialyl Lewis A	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3(Fuc $\alpha$ 1-4)GlcNAc

	CA12	3'-Sialyl Lewis X	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc
	CA13	3'-Sulfated Lewis A	(3S)Gal $\beta$ 1-3(Fuc $\alpha$ 1-4)GlcNAc
	CA14	3'-Sulfated Lewis X	(3S)Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)GlcNAc
	CA15	Blood Group A pentasaccharide type I	GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal
	CA16	Blood group A pentasaccharide type II	GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal
	CA17	Blood Group A tetrasaccharide type VI	GalNAc $\alpha$ 1-3(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4Glc
	CA18	Blood group B hexasaccharide type I	Gal $\alpha$ 1-3-(Fuc $\alpha$ 1-2)Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc
	CA19	Blood group B hexasaccharide type II	Gal $\alpha$ 1-3-(Fuc $\alpha$ 1-2)Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc
	CA20	Blood Group H type I trisaccharide	Fuc $\alpha$ 1-2Gal $\beta$ 1-3GlcNAc
	CA21	Blood Group H type II trisaccharide	Fuc $\alpha$ 1-2Gal $\beta$ 1-4GlcNAc
<b>GSL Glycans (19)</b>	CA22	Gangliotetraose	Gal $\beta$ 1-3GalNAc $\beta$ 1-4Gal $\beta$ 1-4Glc
	CA23	GM1	Gal $\beta$ 1-3GalNAc $\beta$ 1-4(Neu5Ac $\alpha$ 2-3)Gal $\beta$ 1-4Glc
	CA24	Fucosyl GM1	Fuc $\alpha$ 1-2Gal $\beta$ 1-3GalNAc $\beta$ 1-4(Neu5Ac $\alpha$ 2-3)Gal $\beta$ 1-4Glc

	CA25	GM2	Neu5Ac $\alpha$ 2-3(GalNAc $\beta$ 1-4)Gal $\beta$ 1-4Glc
	CA26	GD1a	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GalNAc $\beta$ 1-4(Neu5Ac $\alpha$ 2-3)Gal $\beta$ 1-4Glc
	CA27	GD1b	Neu5Ac $\alpha$ 2-8Neu5Ac $\alpha$ 2-3(Gal $\beta$ 1-3GalNAc $\beta$ 1-4)Gal $\beta$ 1-4Glc
	CA28	GD2	Neu5Ac $\alpha$ 2-8Neu5Ac $\alpha$ 2-3(GalNAc $\beta$ 1-4)Gal $\beta$ 1-4Glc
	CA29	GD3	Neu5Ac $\alpha$ 2-8Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4Glc
	CA30	GT1a	Neu5Ac $\alpha$ 2-8Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GalNAc $\beta$ 1-4(Neu5Ac $\alpha$ 2-3)Gal $\beta$ 1-4Glc
	CA31	GT1c	Gal $\beta$ 1-3GalNAc $\beta$ 1-4(Neu5Ac $\alpha$ 2-8Neu5Ac $\alpha$ 2-8Neu5Ac $\alpha$ 2-3)Gal $\beta$ 1-4Glc
	CA32	GT2	GalNAc $\beta$ 1-4(Neu5Ac $\alpha$ 2-8Neu5Ac $\alpha$ 2-8Neu5Ac $\alpha$ 2-3)Gal $\beta$ 1-4Glc
	CA33	GT3	Neu5Ac $\alpha$ 2-8Neu5Ac $\alpha$ 2-8Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4Glc
	CA34	Forssman antigen pentaose	GalNAc $\alpha$ 1-3GalNAc $\beta$ 1-3Gal $\alpha$ 1-4-Gal $\beta$ 1-4Glc
	CA35	Globo-H hexaose	Fuc $\alpha$ 1-2Gal $\beta$ 1-3GalNAc $\beta$ 1-3Gal $\alpha$ 1-4Gal $\beta$ 1-4Glc
	CA36	Globotriose, Gb3	Gal $\alpha$ 1-4Gal $\beta$ 1-4Glc
	CA37	Globo-N-tetraose, Gb4	GalNAc $\beta$ 1-3Gal $\alpha$ 1-4Gal $\beta$ 1-4Glc

		CA38	Globoisotetraose	GalNAc $\beta$ 1-3Gal $\alpha$ 1-3Gal $\beta$ 1-4Glc
		CA39	Globopentaose (SSEA-3)	Gal $\beta$ 1-3GalNAc $\beta$ 1-3Gal $\alpha$ 1-4Gal $\beta$ 1-4Glc
		CA40	SSEA-4 hexaose	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GalNAc $\beta$ 1-3Gal $\alpha$ 1-4Gal $\beta$ 1-4Glc
<b>HMO (15)</b>		CA41	Lactose	Gal $\beta$ 1-4Glc
		CA42	2'-Fucosyllactose	Fuc $\alpha$ 1-2Gal $\beta$ 1-4Glc
		CA43	3-Fucosyllactose	Gal $\beta$ 1-4(Fuc $\alpha$ 1-3)Glc
		CA44	3'-Sialyllactose	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4Glc
		CA45	6'-Sialyllactose	Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4Glc
		CA46	3'-a-Sialyl-N-acetyllactosamine	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc
		CA47	6'-a-Sialyl-N-acetyllactosamine	Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc
		CA48	Lacto-N-tetraose, LNT	Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc
		CA49	Lacto-N-neotetraose, LNnT	Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc
		CA50	Lacto-N-fucopentaose I	Fuc $\alpha$ 1-2Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc

		CA51	Lacto-N-fucopentaose II	Gal $\beta$ 1-3(Fuca1-4)GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc
		CA52	Lacto-N-fucopentaose III	Gal $\beta$ 1-4(Fuca1-3)GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc
		CA53	LS-tetrasaccharide a (LsTa)	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc
		CA54	LS-tetrasaccharide c (LsTc)	Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc
		CA55	Disialyllacto-N-tetraose (DSLNT)	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3(Neu5Ac $\alpha$ 2-6)GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc
<b>Glucan (10)</b>		CA56	Sophorose	Glc $\beta$ 1-2Glc
		CA57	Gentiobiose	Glc $\beta$ 1-6Glc
		CA58	Nigerose	Glc $\alpha$ 1-3Glc
		CA59	Kojibiose	Glc $\alpha$ 1-2Glc
		CA60	Laminaritriose	Glc $\beta$ 1-3Glc $\beta$ 1-3Glc
		CA61	Maltotetraose	Glc $\alpha$ 1-4Glc $\alpha$ 1-4Glc $\alpha$ 1-4Glc
		CA62	Cellotetraose	Glc $\beta$ 1-4Glc $\beta$ 1-4Glc $\beta$ 1-4Glc
		CA63	1,3:1,4 $\beta$ -Glucotetraose	Glc $\beta$ 1-4Glc $\beta$ 1-3Glc $\beta$ 1-4-Glc




		CA64	6-a-D-Glucopyranosyl maltotriose	Glc $\alpha$ 1-6Glc $\alpha$ 1-4Glc $\alpha$ 1-4Glc
		CA65	Isomaltotetraose	Glc $\alpha$ 1-6Glc $\alpha$ 1-6Glc $\alpha$ 1-6Glc
<b>General Oligosaccharides (53)</b>		CA66	2-O-a-D-Glucopyranosyl-D-galactopyranose	Glc $\alpha$ 1-2Gal
		CA67	2-O-(a-D-Galactopyranosyl)-D-galactopyranose	Gal $\alpha$ 1-2Gal
		CA68	2-O-(b-D-Galactopyranosyl)-D-galactopyranose	Gal $\beta$ 1-2Gal
		CA69	Lactose 3'-sulfate	(3S)Gal $\beta$ 1-4Glc
		CA70	3-O-(a-D-Galactopyranosyl)-D-galactopyranose	Gal $\alpha$ 1-3Gal
		CA71	3-O-(a-D-Galactopyranosyl)-D-glucopyranose	Gal $\alpha$ 1-3Glc
		CA72	3-O-(b-D-Galactopyranosyl)-D-galactopyranose	Gal $\beta$ 1-3Gal
		CA73	3-O-(b-D-Galactopyranosyl)-D-glucopyranose	Gal $\beta$ 1-3Glc
		CA74	4-O-(a-D-Galactopyranosyl)-D-galactopyranose	Gal $\alpha$ 1-4Gal
		CA75	4-O-(a-D-Galactopyranosyl)-D-glucopyranose	Gal $\alpha$ 1-4Glc
		CA76	4-O-(b-D-Galactopyranosyl)-D-galactopyranose	Gal $\beta$ 1-4Gal

	CA77	4-O-( $\beta$ -D-Galactopyranosyl)-D-mannopyranose	Gal $\beta$ 1-4Man
	CA78	6-O-( $\alpha$ -D-Galactopyranosyl)-D-galactopyranose	Gal $\alpha$ 1-6Gal
	CA79	6-O-( $\beta$ -D-Galactopyranosyl)-D-galactopyranose	Gal $\beta$ 1-6Gal
	CA80	6-O-( $\beta$ -D-Galactopyranosyl)-D-glucopyranose	Gal $\alpha$ 1-6Glc
	CA81	3-O-( $\beta$ -D-Galactopyranosyl)-D-arabinose	Gal $\beta$ 1-3Ara
	CA82	Rutinose	Rha $\alpha$ 1-6Glc
	CA83	2-Acetamido-2-deoxy-3-O-( $\alpha$ -D-galactopyranosyl)-D-galactopyranose	Gal $\alpha$ 1-3GalNAc
	CA84	2-Acetamido-2-deoxy-3-O-( $\alpha$ -L-fucopyranosyl)-D-glucopyranose	Fuc $\alpha$ 1-3GlcNAc
	CA85	2-Acetamido-2-deoxy-4-O-( $\alpha$ -D-galactopyranosyl)-D-glucopyranose	Gal $\alpha$ 1-4GlcNAc
	CA86	N-Acetyl-D-lactosamine	Gal $\beta$ 1-4GlcNAc
	CA87	2-Acetamido-2-deoxy-4-O-( $\alpha$ -L-fucopyranosyl)-D-glucopyranose	Fuc $\alpha$ 1-4GlcNAc
	CA88	2-Acetamido-2-deoxy-4-O-( $\beta$ -D-galactopyranosyl)-6-sulfo- $\beta$ -D-glucopyranose	Gal $\beta$ 1-4(6S)GlcNAc
	CA89	2-Acetamido-2-deoxy-6-O-( $\alpha$ -L-	Fuc $\alpha$ 1-6GlcNAc

		fucopyranosyl)-D-glucopyranose	
	CA90	2-Acetamido-2-deoxy-6-O-( $\beta$ -D-galactopyranosyl)-D-galactopyranose	Gal $\beta$ 1-6GalNAc
	CA91	2-Acetamido-3-O-(2-acetamido-2-deoxy- $\alpha$ -D-galactopyranosyl)-2-deoxy-D-galactopyranose	GalNAc $\alpha$ 1-3GalNAc
	CA92	2-O-(2-Acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)-D-mannopyranose	GlcNAc $\beta$ 1-2Man
	CA93	3-O-(2-Acetamido-2-deoxy- $\alpha$ -D-galactopyranosyl)-D-galactopyranose	GalNAc $\alpha$ 1-3Gal
	CA94	3-O-(2-Acetamido-2-deoxy- $\beta$ -D-galactopyranosyl)-D-galactopyranose	GalNAc $\beta$ 1-3Gal
	CA95	3-O-(2-Acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)-D-mannopyranose	GlcNAc $\beta$ 1-3Man
	CA96	6'-Sulfated-N-acetyllactosamine	(6S)Gal $\beta$ 1-4GlcNAc
	CA97	Galacto-N-biose	Gal $\beta$ 1-3GalNAc
	CA98	3'-Sialylgalactose (GM4)	Neu5Ac $\alpha$ 2-3Gal $\beta$
	CA99	6'-Sialylgalactose	Neu5Ac $\alpha$ 2-6Gal
	CA100	3'-Sialylgalacto-N-biose	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GalNAc

	CA10 1	3'- Galactosyllactose	Gal $\beta$ 1-3Gal $\beta$ 1-4Glc
	CA10 2	4'- Galactosyllactose	Gal $\beta$ 1-4Gal $\beta$ 1-4Glc
	CA10 3	6'- Galactosyllactose	Gal $\beta$ 1-6Gal $\beta$ 1-4Glc
	CA10 4	3a,4b- Galactotriose	Gal $\alpha$ 1-3Gal $\beta$ 1-4Gal
	CA10 5	Gala1-3Galb1-4Glc	Gal $\alpha$ 1-3Gal $\beta$ 1-4Glc
	CA10 6	4-O-(6-O-[2- Acetamido-2- deoxy-b-D- glucopyranosyl]-b- D- galactopyranosyl)- D-glucopyranose	GlcNAc $\beta$ 1-6Gal $\beta$ 1-4Glc
	CA10 7	4-O-[3-O-(2- Acetamido-2- deoxy-a-D- galactopyranosyl)- b-D- galactopyranosyl]- D-glucose	GalNAc $\alpha$ 1-3Gal $\beta$ 1-4Glc
	CA10 8	1,2-a-1,2-a-L- Rhamnotriose	Rha $\alpha$ 1-2Rha $\alpha$ 1-2Rha
	CA10 9	4'-O-(2-Acetamido- 2-deoxy-3-O-(b-D- galactopyranosyl)- b-D- galactopyranosyl)- b-D-lactose	Gal $\beta$ 1-3GalNAc $\beta$ 1-4Gal $\beta$ 1-4Glc
	CA11 0	2-O-(a-D- Mannopyranosyl)- D-mannopyranose	Man $\alpha$ 1-2Man
	CA11 1	3-O-(a-D- Mannopyranosyl)- D-mannopyranose	Man $\alpha$ 1-3Man

		CA11 2	4-O-( $\alpha$ -D-Mannopyranosyl)-D-mannose	Man $\alpha$ 1-4Man
		CA11 3	6-O-( $\alpha$ -D-Mannopyranosyl)-D-mannose	Man $\alpha$ 1-6Man
		CA11 4	$\alpha$ -D-Galactosyl-mannotriose	Man $\beta$ 1-4Man $\beta$ 1-4(Gal $\alpha$ 1-6)Man
		CA11 5	$\alpha$ 1-3[ $\alpha$ 1-6] $\alpha$ 1-6[ $\alpha$ 1-3]Mannopentaose	Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man
		CA11 6	1,4-b-D-Mannotetraose	Man $\beta$ 1-4Man $\beta$ 1-4Man $\beta$ 1-4Man
		CA11 7	1,4-b-D-Xylotetraose	Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl $\beta$ 1-4Xyl
		CA11 8	Xyloctaose	 $\dots$ 1-4Xyl $\beta$ 1-4Xyl
<b>N-glycan (28)</b>	<b>Complex</b>	CA11 9		Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-6(Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-
		CA12 0		Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-6(Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-
		CA12 1		Gal $\beta$ 1-4(Fuca1-3)GlcNAc $\beta$ 1-2Man $\alpha$ 1-6(Gal $\beta$ 1-4(Fuca1-3)GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-
		CA12 2		Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4(Fuca1-3)GlcNAc $\beta$ 1-2Man $\alpha$ 1-6(Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4(Fuca1-3)GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-
	<b>Hybrid</b>	CA12 3		Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man $\alpha$ 1-6(GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-
		CA12 4		Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man $\alpha$ 1-6(Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-

	CA12 5		Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man $\alpha$ 1-6(Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-
	CA12 6		Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man $\alpha$ 1-6(Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-
	CA12 7		Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man $\alpha$ 1-6(Gal $\beta$ 1-4(Fuca1-3)GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-
	CA12 8		Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man $\alpha$ 1-6(Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4(Fuca1-3)GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-
<b>High-mannose</b>	CA12 9	NG-016	Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-
	CA13 0	NG-004	Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man $\alpha$ 1-6(Man $\alpha$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-
	CA13 1	NG-003	Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man $\alpha$ 1-6(Man $\alpha$ 1-2Man $\alpha$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-
	CA13 2	NG-001	Man $\alpha$ 1-2Man $\alpha$ 1-6(Man $\alpha$ 1-3)Man $\alpha$ 1-6(Man $\alpha$ 1-2Man $\alpha$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-
	CA13 3	Man-9	Man $\alpha$ 1-2Man $\alpha$ 1-6(Man $\alpha$ 1-2Man $\alpha$ 1-3)Man $\alpha$ 1-6(Man $\alpha$ 1-2Man $\alpha$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-
<b>Bisecting</b>	CA13 4		GlcNAc $\beta$ 1-2Man $\alpha$ 1-6(GlcNAc $\beta$ 1-4)(GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-
	CA13 5		Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-6(GlcNAc $\beta$ 1-4)(Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-
	CA13 6		Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-6(GlcNAc $\beta$ 1-4)(Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-
	CA13 7		Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-6(GlcNAc $\beta$ 1-4)(Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-

		CA138		Gal $\beta$ 1-4(Fuca $\alpha$ 1-3)GlcNAc $\beta$ 1-2Man $\alpha$ 1-6(GlcNAc $\beta$ 1-4)(Gal $\beta$ 1-4(Fuca $\alpha$ 1-3)GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-	
		CA139		Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4(Fuca $\alpha$ 1-3)GlcNAc $\beta$ 1-2Man $\alpha$ 1-6(GlcNAc $\beta$ 1-4)(Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4(Fuca $\alpha$ 1-3)GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-	
	<b>Core-fucose</b>	CA140		GlcNAc $\beta$ 1-2Man $\alpha$ 1-6(GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4(Fuca $\alpha$ 1-6)GlcNAc-	
		CA141		Man $\alpha$ 1-6(GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4(Fuca $\alpha$ 1-6)GlcNAc-	
		CA142		Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-6(GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4(Fuca $\alpha$ 1-6)GlcNAc-	
	<b>LacNAc-extended</b>	CA143	TE024	GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-6(GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc	
		CA144	TE025	Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-6(Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc	
		CA145	TE019	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-6(Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc	
	<b>Glycopeptide</b>	CA146		Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-6(Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-2Man $\alpha$ 1-3)Man $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc-KVANKT	
	<b>O-glycan (14)</b>	<b>Glycoamino acid</b>	CA147	Tn antigen	GalNAc $\alpha$ -Thr
			CA148	STn antigen	Neu5Ac $\alpha$ 2-6GalNAc $\alpha$ -Thr
			CA149	Core 1 (T antigen)	Gal $\beta$ 1-3GalNAc $\alpha$ -Thr
			CA150	ST antigen	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3GalNAc $\alpha$ -Ser

		CA15 1	Core 2	GlcNAc $\beta$ 1-6(Gal $\beta$ 1-3)GalNAc $\alpha$ -Thr
		CA15 2	Core 3	GlcNAc $\beta$ 1-3GalNAc $\alpha$ -Thr
		CA15 3	Core 4	GlcNAc $\beta$ 1-6(GlcNAc $\beta$ 1-3)GalNAc $\alpha$ -Thr
		CA15 4	Core 6	GlcNAc $\beta$ 1-6GalNAc $\alpha$ -Ser
		CA15 5	diST	Neu5Ac $\alpha$ 2-3Gal $\beta$ 1-3(Neu5Ac $\alpha$ 2-6)GalNAc $\alpha$ -Ser
	<b>Glycopeptide</b>	CA15 6	MUC1 Glycopeptide	PPAHGVT(GalNAc)SAPDTRPAPGSTAPPA
		CA15 7	MUC1 Glycopeptide	PPAHGVTS(GalNAc)APDTRPAPGSTAPPA
		CA15 8	MUC1 Glycopeptide	PPAHGVTSAPDT(GalNAc)RPAPGSTAPPA
		CA15 9	MUC1 Glycopeptide	PPAHGVTSAPDTRPAPGS(GalNAc)TAPPA
		CA16 0	MUC1 Glycopeptide	PPAHGVTSAPDTRPAPGST(GalNAc)APPA
<b>GAG</b>	<b>HA</b>	CA16 1	Hyaluronic Acid Polymer (HA93), Mw 93kDa	$\Delta$ GlcA $\beta$ 1,3 [GlcNAc $\beta$ 1,4 GlcA $\beta$ 1,3] <sub>n</sub> GlcNAc
	<b>Heparin</b>	CA16 2	Heparin dp22 (H22)	$\Delta$ UA,2S - GlcNS,6S- [IdoUA,2S - GlcNS,6S] <sub>10</sub>
	<b>CS</b>	CA16 3	Chondroitin Sulphate Oligosaccharide dp20 (CSO20)	$\Delta$ UA - [GalNAc,6S or 4S - GlcA] <sub>9</sub> - GalNAc,6S or 4S



	<b>CSD</b>	CA16 4	Chondroitin Sulphate D Oligosaccharide dp20 (CSDO20)	$\Delta$ UA - [GalNAc,6S or 4S – GlcA +/- 2S] <sub>9</sub> – GalNAc,6S
	<b>DS</b>	CA16 5	Dermatan Sulphate dp20 (DS20)	$\Delta$ UA $\beta$ 1,3 - GalNAc,4S – [IdoA – GalNAc,4S] <sub>9</sub>
	<b>HS</b>	CA16 6	Heparan Sulphate Oligosaccharide dp20 (Hep III, high sulphation)	$\Delta$ UA - GlcNS – [IdoA +/- 2S - GlcNS] <sub>8</sub> – 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  $\mu$ 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 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  $\mu$ g/mL. Calculate the volume of sample needed depending on how many slides and subarrays are to be assayed. **We recommend using 100  $\mu$ L sample per well for 16 subarray cassettes and 200  $\mu$ L 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  $\mu$ L per well for 16 subarray cassettes and 80  $\mu$ L for 8 subarray cassettes. We caution that using a minimal volume in the wells has an increased risk of the array drying out during the assay and may also cause unequal distribution of the sample across the arrayed surface which may result in signal variation. Please ensure each sample is homogeneous and thoroughly mixed.

## Assay Protocol

### Part 1 – Blocking

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

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

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

### Part 2 – Binding Assay

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

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

### Part 3 – Wash

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

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

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

1. Unless the secondary biotinylated antibody sample is bacteria or cells, centrifuge samples briefly to avoid adding irrelevant particles to the array.

2. After removing the third GAAB wash, immediately add the secondary biotinylated antibody to each well. Seal the wells with adhesive film and incubate on the shaker for 1 hour at 80 rpm. Shaking at a faster speed can prevent protein aggregation. Longer incubation time is acceptable, but not necessary.
3. After incubation, repeat Part 3 – Wash.

#### Part 5 – Fluorescent Staining

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

#### Part 6 – Final Wash and Dry

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

### **Analysis**

Scan the slide in a laser fluorescence scanner at the wavelength of emission for the fluorophore used. Adjust the laser power and PMT to obtain the highest possible signals without being saturated (saturated positive control signal is okay). Analyze data with microarray analysis software. If there is specific binding, the signal intensity should be higher than the background signal (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. 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.

Interpretation of 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.

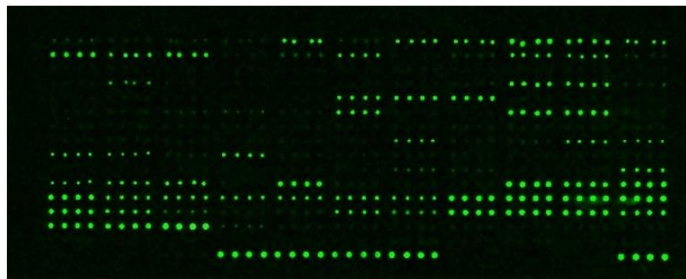
**Marker:** The array marker should show a fluorescence signal regardless of the assay. It is there primarily to aid with the orientation of the array map during analysis.

**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.

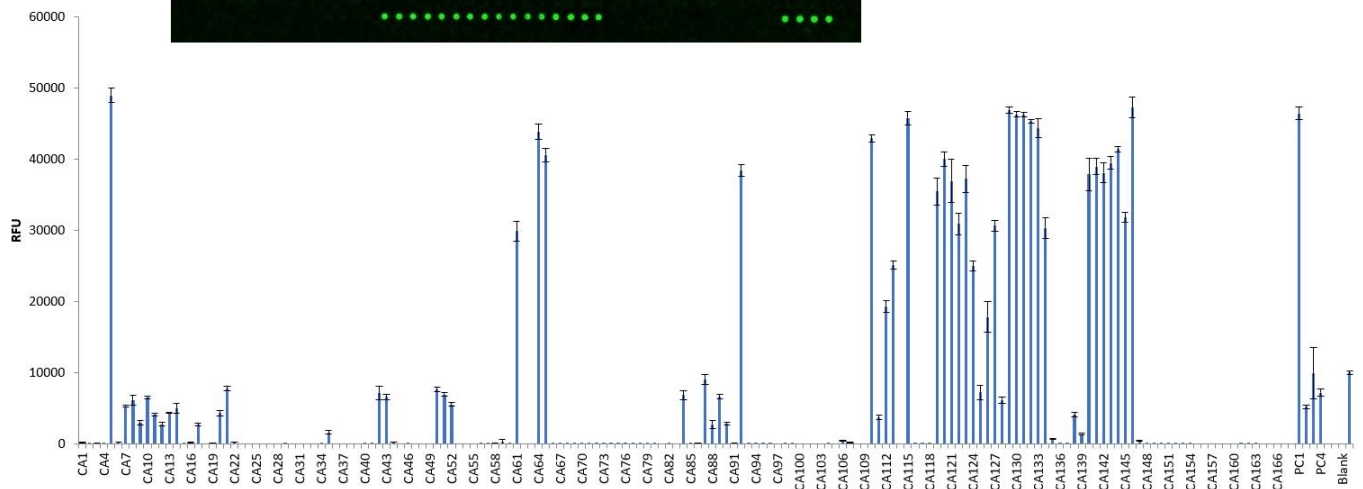
### Typical Binding Assay Result from the CatchAll Glycan Array

The CatchAll glycan array was assayed with biotinylated Concanavalin A (Con A) (10  $\mu\text{g/mL}$ ), 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	<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>