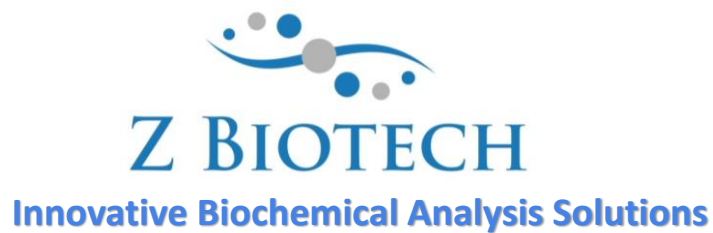


Poly-LacNAc Glycan Microarray User Manual



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Introduction

Poly-N-acetyl-lactosamine (poly-LacNAc) structures represent a distinct class of linear glycan structures, comprising repeating disaccharide units of N-acetylglucosamine (GlcNAc) and galactose (Gal). These units are interconnected via a $\beta(1,3)$ linkage between GlcNAc and Gal, and a $\beta(1,4)$ linkage between Gal and the succeeding GlcNAc. Such structures are typically integrated as disaccharide repeats in O-glycans, N-glycans, and glycolipids, constituting components of their elongated or branched side chains. These glycan extensions serve as backbone polymers, enabling further glycan branching to form I-antigen structures. Moreover, they may undergo modifications such as sulfation, resulting in the production of keratan sulfate or the incorporation of specific terminal capping structures. Examples of these terminal capping structures include ABO, Lewis blood group, and HNK-1 antigen structures.

LacNAc structures hold considerable importance in numerous biological processes, owing to their participation in cell-cell interactions, cell signaling, and immune recognition. These structures contribute to cell adhesion by engaging with lectins, carbohydrate-binding proteins that facilitate cell-cell communication. Such interactions have an impact on cell signaling pathways, migration, and differentiation. Furthermore, LacNAc structures serve as ligands for immune receptors, including selectins, which play a crucial role in leukocyte rolling and adhesion during inflammatory responses. They can also be recognized by galectins, a family of lectins that modulate immune cell activation, differentiation, and apoptosis. Certain pathogens, such as bacteria and viruses, can bind to LacNAc structures present on host cell surfaces, thereby enabling infection. To counteract this, host organisms have evolved strategies to modify LacNAc structures, effectively preventing pathogen attachment and invasion.

LacNAc structures, while essential for maintaining biological homeostasis, have been implicated in various diseases due to their abnormal expression. Altered LacNAc expression has been linked to chronic inflammation and autoimmune disorders, including rheumatoid arthritis and systemic lupus erythematosus. Modifications in LacNAc structures affect immune cell interactions, activating pro-inflammatory pathways and contributing to the onset and progression of these conditions.

Recent studies have demonstrated that LacNAc structures within N-glycans protect tumor cells from T cell immunosurveillance by disrupting proper immunological synapse formation and diminishing transcriptional activation, cytokine production, and cytotoxicity. These findings suggest that LacNAc structures on tumor cells play a critical role in modulating the quality and magnitude of T-cell responses. This knowledge opens avenues for future research into the potential of cancer-associated LacNAc structures as immunotherapy targets. By selectively targeting abnormal LacNAc structures expressed by cancer cells, it may be feasible to enhance anti-tumor immune responses and develop more effective cancer treatments.

As the significance of LacNAc structures in human health and disease becomes increasingly apparent, Z Biotech has developed a robust poly-LacNAc microarray platform to expedite research on the interactions between poly-LacNAc structures and diverse biological samples. The poly-LacNAc array comprises 77 unique structures, incorporating both linear and branched poly-LacNAc structures. These closely related isoforms, characterized by well-defined repeat units, offer a comprehensive set of poly-LacNAc structures for exploring mammalian and microbial poly-LacNAc-binding proteins.

The array system boasts 8 or 16 identical subarrays, enabling simultaneous analysis of multiple samples. With an uncomplicated assay format that necessitates only a small sample volume, the platform yields reliable glycan-binding information at high throughput. This cutting-edge tool holds the potential to augment our understanding of LacNAc structures and their implications across various biological contexts.

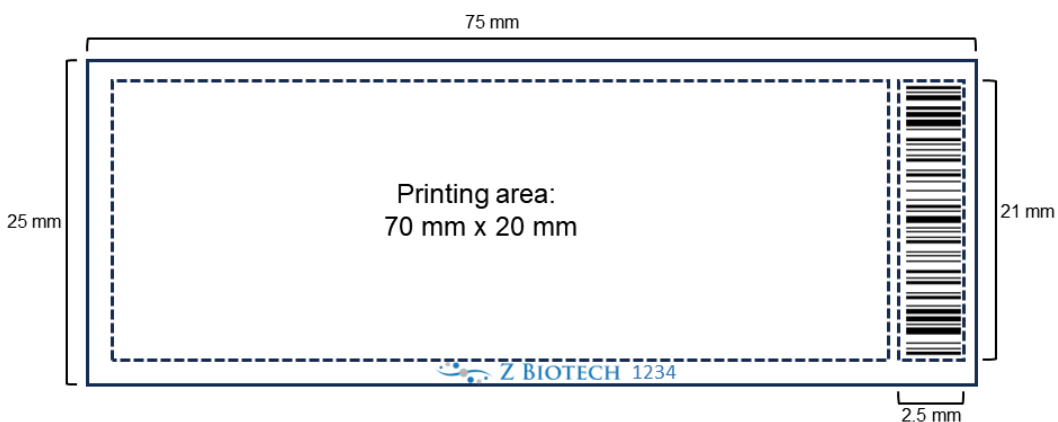
Handling and Storage

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.

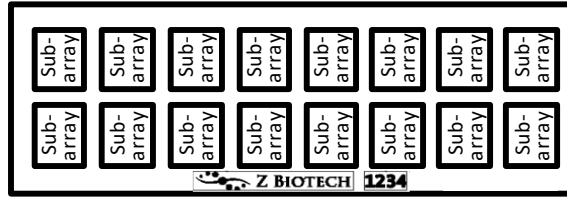
Array Map/Schematic

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



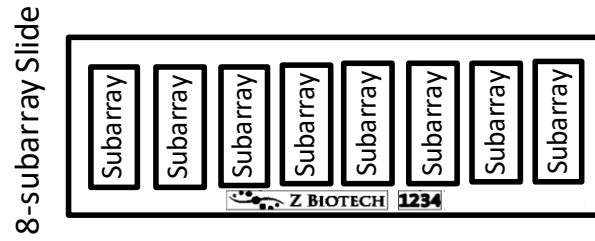
Array Map (16-subarray slides):

16-subarray Slide



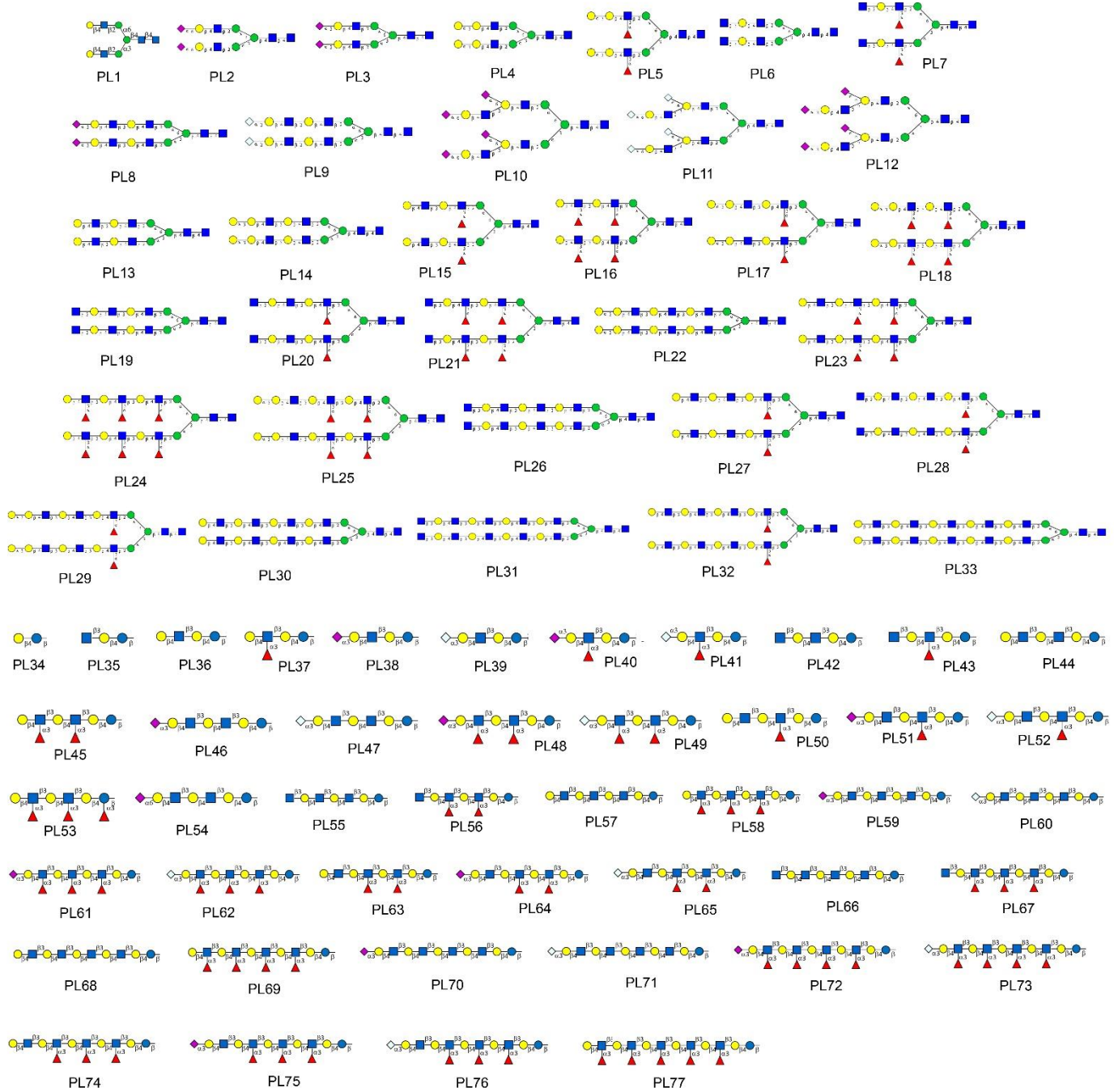
PL1	PL1	PL1	PL2	PL2	PL2	PL3	PL3	PL3	PL4	PL4	PL4	PL5	PL5	PL5
PL6	PL6	PL6	PL7	PL7	PL7	PL8	PL8	PL8	PL9	PL9	PL9	PL10	PL10	PL10
PL11	PL11	PL11	PL12	PL12	PL12	PL13	PL13	PL13	PL14	PL14	PL14	PL15	PL15	PL15
PL16	PL16	PL16	PL17	PL17	PL17	PL18	PL18	PL18	PL19	PL19	PL19	PL20	PL20	PL20
PL21	PL21	PL21	PL22	PL22	PL22	PL23	PL23	PL23	PL24	PL24	PL24	PL25	PL25	PL25
PL26	PL26	PL26	PL27	PL27	PL27	PL28	PL28	PL28	PL29	PL29	PL29	PL30	PL30	PL30
PL31	PL31	PL31	PL32	PL32	PL32	PL33	PL33	PL33	PL34	PL34	PL34	PL35	PL35	PL35
PL36	PL36	PL36	PL37	PL37	PL37	PL38	PL38	PL38	PL39	PL39	PL39	PL40	PL40	PL40
PL41	PL41	PL41	PL42	PL42	PL42	PL43	PL43	PL43	PL44	PL44	PL44	PL45	PL45	PL45
PL46	PL46	PL46	PL47	PL47	PL47	PL48	PL48	PL48	PL49	PL49	PL49	PL50	PL50	PL50
PL51	PL51	PL51	PL52	PL52	PL52	PL53	PL53	PL53	PL54	PL54	PL54	PL55	PL55	PL55
PL56	PL56	PL56	PL57	PL57	PL57	PL58	PL58	PL58	PL59	PL59	PL59	PL60	PL60	PL60
PL61	PL61	PL61	PL62	PL62	PL62	PL63	PL63	PL63	PL64	PL64	PL64	PL65	PL65	PL65
PL66	PL66	PL66	PL67	PL67	PL67	PL68	PL68	PL68	PL69	PL69	PL69	PL70	PL70	PL70
PL71	PL71	PL71	PL72	PL72	PL72	PL73	PL73	PL73	PL74	PL74	PL74	PL75	PL75	PL75
PL76	PL76	PL76	PL77	PL77	PL77	NC	NC	NC	PC1	PC1	PC1	PC2	PC2	PC2
PC3	PC3	PC3	PC4	PC4	PC4	Blank	Blank	Blank	Blank	Blank	Blank	M	M	M

Array Map (8-subarray slides):



PL1	PL1	PL1	PL2	PL2	PL2	PL3	PL3	PL3	PL4	PL4	PL4	PL5	PL5	PL5
PL6	PL6	PL6	PL7	PL7	PL7	PL8	PL8	PL8	PL9	PL9	PL9	PL10	PL10	PL10
PL11	PL11	PL11	PL12	PL12	PL12	PL13	PL13	PL13	PL14	PL14	PL14	PL15	PL15	PL15
PL16	PL16	PL16	PL17	PL17	PL17	PL18	PL18	PL18	PL19	PL19	PL19	PL20	PL20	PL20
PL21	PL21	PL21	PL22	PL22	PL22	PL23	PL23	PL23	PL24	PL24	PL24	PL25	PL25	PL25
PL26	PL26	PL26	PL27	PL27	PL27	PL28	PL28	PL28	PL29	PL29	PL29	PL30	PL30	PL30
PL31	PL31	PL31	PL32	PL32	PL32	PL33	PL33	PL33	PL34	PL34	PL34	PL35	PL35	PL35
PL36	PL36	PL36	PL37	PL37	PL37	PL38	PL38	PL38	PL39	PL39	PL39	PL40	PL40	PL40
PL41	PL41	PL41	PL42	PL42	PL42	PL43	PL43	PL43	PL44	PL44	PL44	PL45	PL45	PL45
PL46	PL46	PL46	PL47	PL47	PL47	PL48	PL48	PL48	PL49	PL49	PL49	PL50	PL50	PL50
PL51	PL51	PL51	PL52	PL52	PL52	PL53	PL53	PL53	PL54	PL54	PL54	PL55	PL55	PL55
PL56	PL56	PL56	PL57	PL57	PL57	PL58	PL58	PL58	PL59	PL59	PL59	PL60	PL60	PL60
PL61	PL61	PL61	PL62	PL62	PL62	PL63	PL63	PL63	PL64	PL64	PL64	PL65	PL65	PL65
PL66	PL66	PL66	PL67	PL67	PL67	PL68	PL68	PL68	PL69	PL69	PL69	PL70	PL70	PL70
PL71	PL71	PL71	PL72	PL72	PL72	PL73	PL73	PL73	PL74	PL74	PL74	PL75	PL75	PL75
PL76	PL76	PL76	PL77	PL77	PL77	NC	NC	NC	PC1	PC1	PC1	PC2	PC2	PC2
PC3	PC3	PC3	PC4	PC4	PC4	Blank	Blank	Blank	Blank	Blank	Blank	M	M	M

Poly-LacNAc Glycan Array SNFG Structures:

**Symbols:**

 Man
  GlcNAc
  Gal
  L-Fuc
  Neu5Ac
  Neu5Gc

Identification List:

ID	Structure
PL1	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 1-4GlcNAc
PL2	Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2Man α 1-6(Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
PL3	Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-2Man α 1-6(Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
PL4	Gal α 1-3Gal β 1-4GlcNAc β 1-2Man α 1-6(Gal α 1-3Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
PL5	Gal α 1-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-2Man α 1-6(Gal α 1-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
PL6	GlcNAc β 1-3Gal β 1-4GlcNAc β 1-2Man α 1-6(GlcNAc β 1-3Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
PL7	GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-2Man α 1-6(GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
PL8	Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-2Man α 1-6(Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
PL9	Neu5Gc α 2-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-2Man α 1-6(Neu5Gc α 2-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
PL10	Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-3(Neu5Ac α 2-6)Gal β 1-4GlcNAc β 1-2Man α 1-6(Neu5Ac α 2-6Gal β 1-4GlcNAc β 1-3(Neu5Ac α 2-6)Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
PL11	Neu5Gc α 2-6Gal β 1-4GlcNAc β 1-3(Neu5Gc α 2-6)Gal β 1-4GlcNAc β 1-2Man α 1-6(Neu5Gc α 2-6Gal β 1-4GlcNAc β 1-3(Neu5Gc α 2-6)Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
PL12	Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-3(Neu5Ac α 2-6)Gal β 1-4GlcNAc β 1-2Man α 1-6(Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-3(Neu5Ac α 2-6)Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
PL13	Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-2Man α 1-6(Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
PL14	Gal α 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-2Man α 1-6(Gal α 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
PL15	Gal β 1-4GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-2Man α 1-6(Gal β 1-4GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
PL16	Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-2Man α 1-6(Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc

PL30	Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-2Man α 1-6(Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
PL31	GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-2Man α 1-6(GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
PL32	Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-2Man α 1-6(Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
PL33	Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-2Man α 1-6(Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc
PL34	Gal β 1-4Glc
PL35	GlcNAc β 1-3Gal β 1-4Glc
PL36	Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc
PL37	Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc
PL38	Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc
PL39	Neu5Gc α 2-3Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc
PL40	Neu5Ac α 2-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc
PL41	Neu5Gc α 2-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc
PL42	GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc
PL43	GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc
PL44	Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc
PL45	Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc
PL46	Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc
PL47	Neu5Gc α 2-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4Glc
PL48	Neu5Ac α 2-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc
PL49	Neu5Gc α 2-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc
PL50	Gal β 1-4GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc
PL51	Neu5Ac α 2-3Gal β 1-4GlcNAc β 1-3Gal β 1-4(Fuc α 1-3)GlcNAc β 1-3Gal β 1-4Glc

PL52	Neu5Gca2-3Galβ1-4GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4Glc
PL53	Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)Glc
PL54	Neu5Aca2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc
PL55	GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc
PL56	GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4Glc
PL57	Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc
PL58	Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4Glc
PL59	Neu5Aca2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc
PL60	Neu5Gca2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc
PL61	Neu5Aca2-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4Glc
PL62	Neu5Gca2-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4Glc
PL63	Galβ1-4GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4Glc
PL64	Neu5Aca2-3Galβ1-4GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4Glc
PL65	Neu5Gca2-3Galβ1-4GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4Glc
PL66	GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc
PL67	GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4Glc
PL68	Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc
PL69	Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4Glc
PL70	Neu5Aca2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc
PL71	Neu5Gca2-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc
PL72	Neu5Aca2-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4Glc

PL73	Neu5Gca2-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4Glc
PL74	Galβ1-4GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4Glc
PL75	Neu5Aca2-3Galβ1-4GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4Glc
PL76	Neu5Gca2-3Galβ1-4GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4Glc
PL77	Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4(Fuca1-3)GlcNAcβ1-3Galβ1-4Glc

Controls

NC: Negative control, Print Buffer

PC1: Positive control 1, a biotinylated probe (0.01 mg/ml)

PC2: Postitive control 2, Human IgG (0.1 mg/ml)

PC3: Postitive control 3, Mouse IgG (0.1 mg/ml)

PC4: Postitive 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

Part 1 – Blocking

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

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

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

Part 2 – Binding Assay

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

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

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

Part 3 – Wash

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

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

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

1. Unless the secondary biotinylated antibody sample is bacteria or cells, centrifuge samples briefly to avoid adding irrelevant particles to the array.
2. After completely removing the third GAAB wash, immediately add the secondary biotinylated antibody to each well. Seal the wells with adhesive film and incubate on the shaker for 1 hour at 80 rpm. Shaking at a faster speed can prevent protein aggregation. Longer incubation time is acceptable, but not necessary.
3. After incubation repeat Part 3 – Wash.

Part 5 – Fluorescent Staining

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

Part 6 – Final Wash and Dry

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

Analysis

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

Interpretation of Control Signals:

Negative 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 fluorescence signal regardless of the assay. It is there primarily to aid with orientation of the array map during analysis.

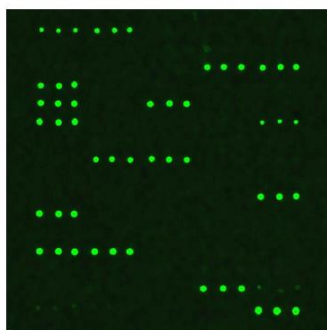
Biotinylated Mannose (PC1): This positive control will bind directly to the fluorescent labelled streptavidin. If your glycan-binding protein sample is already fluorescently labelled, or in any case where the addition of fluorescently labelled streptavidin to the array was not performed (Part 5 – Fluorescent staining) this positive control will not be reactive.

IgG (PC2, PC3, PC4): IgG is an antibody found in blood that is a primary component of humoral immunity. If the glycan-binding or secondary antibody sample is an anti-IgG from human, rabbit, or mouse it should bind to the respective IgG control.

Typical Binding Assay Result from the Poly-LacNAc Microarray

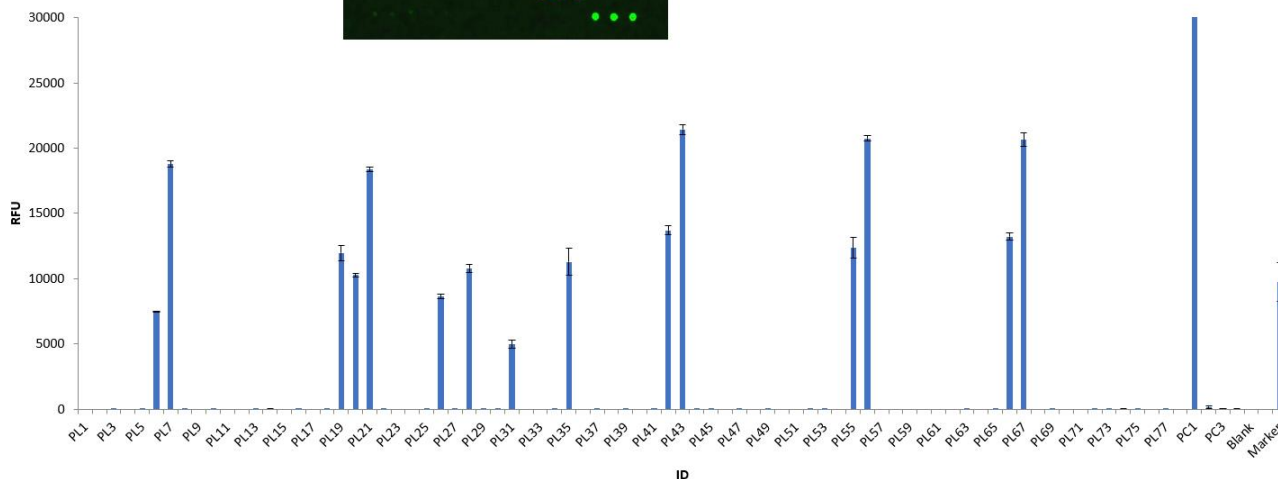
Lectin GS-II, originating from the seeds of the tropical African legume *Griffonia simplicifolia*, is a 113,000-dalton protein comprising identical subunits. In this assay, the binding specificity of GS-II lectin was evaluated using a Poly-LacNAc array.

The Poly-LacNAc array was treated with biotinylated GS-II lectin (10 $\mu\text{g}/\text{mL}$), followed by the application of streptavidin (Cy3). The array was subsequently scanned using a microarray scanner at a wavelength of 532 nm. The positive control exhibited binding signals as anticipated. GS-II was found to bind to poly-LacNAc chain structures terminated with GlcNAc specifically.



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