

RayBio® Glycan Array 300

Patent Pending Technology User Manual (revised Jan. 20th, 2025)

Identification of the specific glycan binding proteins in serum, plasma, cell culture supernatants, cell/tissue lysates or other body fluids.

Cat# GA-Glycan-300-1 (4 Sample Kit)

Cat# GA-Glycan-300-2 (8 Sample Kit)

Cat# GA-Glycan-300-4 (16 Sample Kit)

**Please read manual carefully
before starting experiment**



Your Provider of Excellent Protein Array Systems and Services

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I. Introduction

Glycocalyx, literally meaning ‘sugar coat’, is an extracellular polymeric coating surrounding many prokaryotic and eukaryotic cells consisting of glycoproteins, glycolipids, proteoglycans and glycosaminoglycans. The constituents of the glycocalyx play an important role in the process of protein folding and trafficking, cell signaling and intercellular interactions, cell pathogen interactions, and immunity. However, detection tools for the research of glycobiology are currently in very limited supply.

Raybiotech has pioneered the development of antibody arrays which are now widely applied in the research community with thousands of peer reviewed publications, including in Cell and Nature. Taking advantage of advancements in microarray technology developed for antibody arrays, we have developed a new generation glycan array, the Glycan-300, soon after previously launching the Glycan-100. The Glycan-300 is currently the largest commercially available glycan array for screening protein-carbohydrate interactions. This array will help researchers: 1) identify glycan binding partners in biological samples, 2) identify whether target proteins are carbohydrate binding proteins, 3) probe binding of pathogens including bacteria, viruses and whole cells to glycans,¹⁻³ 4) profile the substrate specificity of enzymes (glycosyltransferases, glycosidases, etc.)⁴⁻⁶ and 5) profile the inflammatory and/or immune response.

The 300 synthetic glycans featured in the new generation Glycan Glycan-300 array consists of four groups of targets including the 100 glycans from the Raybio Glycan-100 array and an additional 80 N-glycans, 50 glycolipid glycans, and 50 human milk oligosaccharide glycans. The included glycans have been frequently identified as

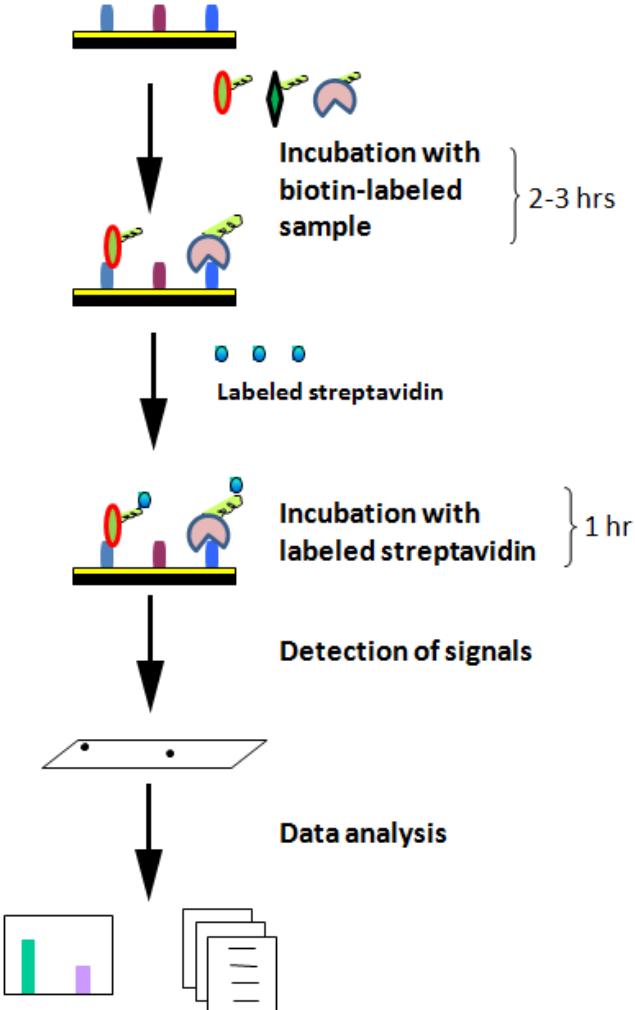
structures showing important binding functions in the literature. For example, a variety of sialosides have been shown to bind to the influenza virus with a serotype-specific pattern.⁷⁻⁹ Galectins, which are involved in apoptosis, cell adhesion and T-cell activation suppression, function by binding beta-galactosides.¹⁰⁻¹² Glycosphingolipids interact with growth factor receptors to modify signal transduction or with bacterial toxin subunits, such as Vero(Shiga) toxin, cholera toxin, and the heat labile E.coli toxins, to induce cell apoptosis¹³. Additionally, oligosaccharides from human milk bind to microbes to promote growth of normal flora and inhibit pathogens in the gastrointestinal tract¹⁴. This array provides a powerful tool for researchers in glycobiology and other fields.

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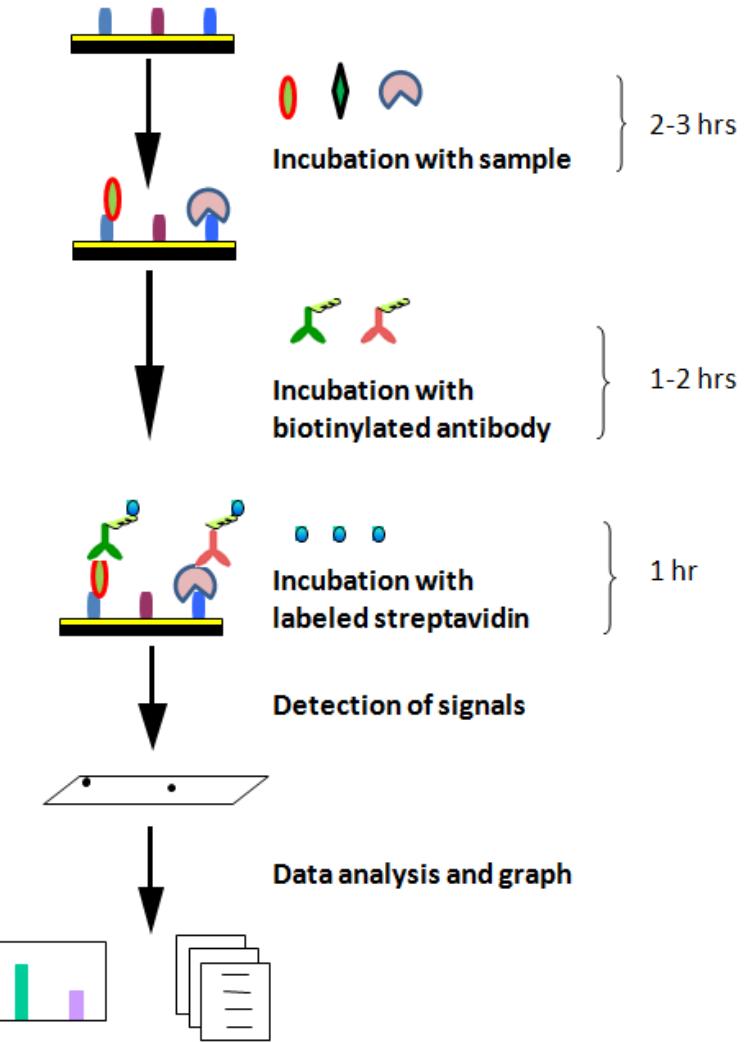
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How It Works

Label-based Procedure



Sandwich-based Procedure



II. Materials Provided

Upon receipt, all components of the RayBiotech Glycan Array 300 kit should be stored at -20°C. After initial use, remaining reagents should be stored at 4°C to avoid repeated freeze-thaw cycles and may be stored for up to 3 months (Labeling Reagent, Item B, should be prepared fresh each time before use). Unused glass slides should be kept at -20 °C and repeated freeze-thaw cycles should be avoided (slides may be stored for up to 6 months). The entire kit should be used within 6 months of purchase.

Components

Item	Description	1-Slide kit	2-Slide kit
A	Dialysis Vials and Floating Dialysis Rack	8 vials/1 rack	16 vials/1 rack
B	Labeling Reagent	1 vial	2 vials
D	Stop Solution	1 vial (50ul)	1 vial (50ul)
E	Glycan Array Glass Slide Assembly*	1 Slide	2 Slides
F	Sample Diluent	1 bottle (8ml)	2 bottles (2x8ml)
G	20X Wash Buffer I	1 bottle (30ml)	1 bottle (30ml)
H	20X Wash Buffer II	1 bottle (30ml)	1 bottle (30ml)
I	Cy5 equivalent dye-conjugated Streptavidin	2 vials	4 vials
J	Adhesive device sealer	2	4
K	Labeling Buffer	1 bottle (30ml)	1 bottle (30ml)
M	Slide Washer/Dryer	1	1
N	Manual	1	1

* Each slide contains 4 identical subarrays

Additional Materials Required

- Detection antibodies of interest (For sandwich-based method only)
- Distilled or de-ionized water
- Orbital shaker
- Laser scanner for fluorescence detection
- Aluminum foil
- Small plastic or glass containers
- 1.5 mL Polypropylene microcentrifuge tubes

- KCl, NaCl, KH₂PO₄ and Na₂HPO₄ (For label-based method only)
- Beaker, stir plate and stir bar
- Pipettors, pipette tips and other common lab consumables

III. General Considerations

A. Label-Based vs. Sandwich-Based Method

The RayBiotech Glycan Array 300 Kit can be used with either a label-based method or with a sandwich-based method.

- In the label-based method, the proteins or antibodies in the sample are biotin labeled (via a simple reaction targeting primary amines), allowing direct detection on the array via a Cy5 equivalent dye-conjugated biotin-streptavidin complex. A complete protocol and the primary materials for this procedure are included with the kit.
- The sandwich-based method is used for antibody-based detection of target proteins captured on the array. The user will need to supply the labeled reporter antibodies specific for their protein of interest. An example protocol for this procedure with a general “Antibody Cocktail” is included in this manual. Specific antibody concentrations and conditions will need to be determined by the end user.

B. Preparation of Samples

- We recommend the following parameters for your samples:
 - 400 to 800 µl of 40X diluted serum, plasma, cell culture media, or other body fluid, or 50-500 µg/ml of protein for cell and tissue lysates.

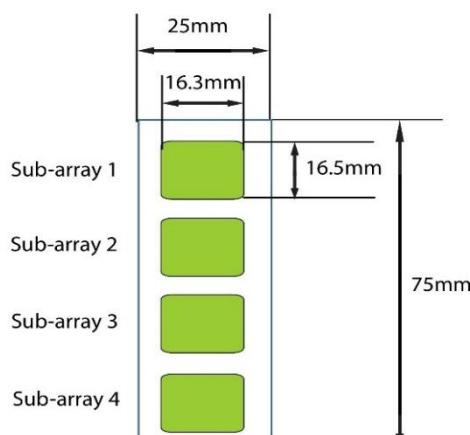
Note: If you experience high background or the readings exceed the detection range, further dilution of your sample is recommended.

C. Handling Glass Slides

- The microarray slides are delicate. Please do not touch the surface of the slides with pipette tips, forceps or your fingers. Hold the slides by the edges only.
- Handle the slides with latex free gloves in a clean environment.
- Do not remove the glass slide from the chamber assembly until step 21, and take great care not to break the glass slide when doing so.
- Permanent marker ink can significantly interfere with fluorescent signal detection. Never mark anywhere on the front (arrayed) side of the slide. It's best to avoid using marker completely, however if you need to number the slide, please add a small mark only on the back of the slide along the top or bottom edge using a green or blue ultra-fine point Sharpie® brand marker, only after the slide is completely dry.
- Remove reagents/sample by gently applying suction with a pipette to corners of each chamber. Do not touch the printed area of the array, only the sides.

D. Layout of Glycan Array 300 Glass Slide

Four identical sub-arrays on one slide



4 printed sub-arrays per glass chip

E. Incubations and Washes

- Cover the incubation chamber with adhesive film during incubation to prevent evaporation, particularly when incubation is more than 2 hours.
- Avoid foaming during incubation steps and wash steps. Be sure to remove all bubbles from the sub-array surface.
- Perform all incubation and wash steps with gentle rocking motion (~0.5 to 1 cycle/sec).
- Avoid cross-contamination of samples to neighboring wells. To remove Wash Buffers and other reagents from chamber wells, you may invert the Glass Slide Assembly to decant, and aspirate the remaining liquid.
- Several incubation steps such as step 11 (sample incubation), step 15 (detection antibody incubation), may be done overnight at 4°C. Please make sure to cover the incubation chamber tightly to prevent evaporation.
- Unlike most Cy5 fluors, the Streptavidin-Conjugated Fluor used in this kit is very stable at room temperature (RT) and resistant to photobleaching on the hybridized glass slides. However, please protect glass slides from strong direct light and temperatures above RT.

IV. Protocol

READ ENTIRE PROTOCOL BEFORE STARTING

Note: Biotin Label-Based protocol starts here. For the Sandwich-Based protocol (using researcher's own detection antibody), start at section C. Dry the Glass Slide, step 8, on page 11. Do not do steps 1-7.

A. Dialysis of Sample

Note: Samples must be dialyzed prior to biotin-labeling (Steps 5–7).

1. Prepare enough dialysis buffer (1X PBS, pH=8.0) for all dialysis steps herein and after. To prepare 1 L dialysis buffer, dissolve 0.2 g KCl, 8 g

NaCl, 0.2 g KH₂PO₄ and 1.15 g Na₂HPO₄ in 800 ml ddH₂O. Adjust pH=8.0 with 1M NaOH and adjust final volume to 1000 ml with ddH₂O.

2. Add each sample into a separate Dialysis Tube (Item A). Load 200 µl cell culture supernatant or 100 µl cell or tissue lysate (1~2 mg/ml total protein) or 20 µl serum or plasma + 80 µl 1X PBS, pH=8 (5-fold dilution). Carefully place Dialysis Tubes into Floating Dialysis Rack (Item A).

Note: If the samples appear to be cloudy, transfer the samples to a clean tube, centrifuge at 13,000 rpm for 20 minutes at 2-8°C. If the samples are still not clear, store them at -20°C for 20 minutes. Remove from the freezer, immediately centrifuge at 13,000 rpm for 20 minutes at 2-8°C.

3. Place Floating Dialysis Rack into at least 500 ml dialysis buffer in a large beaker. For more than 2 samples, make certain to use at least 300 ml dialysis buffer for each sample (more buffer will improve the efficiency of dialysis). Place beaker on a stir plate and dialyze for at least 3 hours at 4°C, stirring buffer gently. Then exchange the dialysis buffer and repeat dialysis for another 3 hours at 4°C. Transfer dialyzed sample to a clean eppendorf tube. Spin dialyzed samples for 5 min at 10,000 rpm to remove any particulates or precipitants, and then transfer the supernatants to a clean tube.

Note: The sample volume may change during dialysis.

Note: Dialysis procedure may proceed overnight.

Note: Determine the total protein concentration for cell culture supernatants or cell/tissue lysate after dialysis procedure (Step 3). We recommended using a BCA total protein assay (eg, RayBiotech, Catalog # 68QT-BCAPro-S1000).

B. Biotin-labeling Sample

Note: Amines (e.g., Tris, glycine) and azides quench the biotinylation reaction. Avoid contaminating samples with these chemicals prior to biotinylation.

4. Immediately before use, prepare 1X Labeling Reagent. Briefly spin down the Labeling Reagent tube (Item B). Add 100 μ l 1X PBS into the tube, pipette up and down or vortex slightly to dissolve the lyophilized reagent.
5. Add 1X Labeling Reagent to dialyzed samples.
 - a. For labeling cell culture supernatants: transfer 180 μ l dialyzed sample into a new tube. Add 36 μ l of 1X Labeling Reagent Solution per 1 mg total protein in dialyzed cell culture supernatant. Mix well. For example, if sample's total protein concentration is 0.5 mg/ml you need to add 3.24 μ l 1X Labeling Reagent to the tube of 180 μ l dialyzed sample.

Note: You need to biotin-label 360 μ l of dialyzed sample if dilution of the biotin-labeled samples is 2 fold in step 11 on page 12.

- b. For labeling serum or plasma: Add 22 μ l of 1X Labeling Reagent Solution into a new tube containing 35 μ l dialyzed serum or plasma sample and 155 μ l Labeling Buffer (Item K).
- c. For labeling cell or tissue lysates: transfer 30 μ g (15 μ l of 2 mg/ml) cell or tissue lysates into a tube and add Labeling Buffer (Item K) for a total volume of 300 μ l. Then add 3.3 μ l of 1X Labeling Reagent Solution.

Note: To normalize serum/plasma or cell/tissue lysate concentrations during biotinylation, measure sample volume before and after dialysis. Then adjust the volumes of dialyzed serum/plasma or cell/tissue lysates and Labeling Buffer to compensate. For example, if the sample volume doubles after dialysis, then use twice as much serum/plasma in the labeling reaction (70 μ l) and reduce the Labeling Buffer to 120 μ l.

6. Incubate the reaction solution at room temperature with gentle rocking or shaking for 30 min. Mix the reaction solution by gently tapping the tube every 5 min.
7. Add 3 μ l Stop Solution (Item D) into each reaction tube and immediately dialyze as directed in Step 3.

Note: Biotinylated samples can be stored at -20°C or -80°C until you are ready to proceed with the assay.

C. Dry the Glass Slide

Note: Sandwich-Based protocol starts here.

8. Take out the package containing the Glycan Array Glass Slide Assembly (Item E) and let the slide equilibrate to room temperature inside the sealed plastic bag for 20-30 minutes. Remove slide from the plastic bag; peel off the cover film, and let it air dry at room temperature for another 1-2 hours. Do not disassemble the Glass Slide from the chamber assembly.

Note: Protect the slide from dust or other contaminants.

D. Blocking and Incubation

Note: Glass slide should be completely dry before adding Sample Diluent to wells.

9. Block sub-arrays by adding 400 µl Sample Diluent (Item F) into each well and incubate at room temperature for 30 min. Ensure there are no bubbles on the array surface.
10. Immediately prior to sample incubation, spin biotin-labeled samples for 5 min at 10,000 rpm to remove any particulates or precipitates. Dilute samples with Sample Diluent (Item F).

Note: Recommended dilution of the biotin-labeled samples with Sample Diluent prior to incubation is 2-10 fold for cell culture supernatants, 20-fold for serum/plasma or 30 fold cell/tissue lysate.

11. Decant buffer from each well. Add 400 µl of sample to each well. Incubate arrays with gentle rocking or shaking at room temperature for 2-3 hours. (Longer incubation time is preferable if higher signal intensity is desired)

Note: This step may be done overnight at 4°C for highest intensities.

12. Wash:

- a. Based on number of samples and remaining protocol, calculate the amounts of 1x Wash Buffers I & II that are needed for each step of the protocol. Separately dilute required amounts of 20x Wash Buffer I and 20x Wash Buffer II with ddH₂O to 1x concentration. *For example if 12 ml of 1x Wash Buffer I is needed then 600 µl of 20x Wash Buffer I would be diluted to a final volume of 12 ml.*
- b. Decant the samples from each well, and wash each well 5 times (5 min each) with 800 µl of 1x Wash Buffer I at room temperature with gentle shaking. Completely remove wash buffer between each wash step.
- c. (*Optional for Cell and Tissue Lysates*) Put the glass slide with frame into a box with 1x Wash Buffer I (cover the whole glass slide and frame with Wash Buffer I), and wash at room temperature with gentle shaking for 20 min.
- d. Decant the 1x Wash Buffer I from each well, wash 2 times (5 min each) with 800 µl of 1x Wash Buffer II at room temperature with gentle shaking. Completely remove wash buffer between each wash step.

Note: Incomplete removal of the wash buffer after each wash step may cause “dark spots”. (i.e., background signal higher than that of the spot.)

E. Incubation with Biotinylated Detection Antibody (provided by researcher)

Note: For the Label-Based protocol, go directly to F. Incubation with Cy5 Equivalent Dye-Streptavidin, step 17, on page 13. Do not do steps 13-16.

13. If the researcher wishes to use their own antibody to detect specific bound proteins, we recommend using a biotinylated antibody at a dilution appropriate for Western blot. Optimal dilution must be determined by the researcher.
14. Dilute the detection antibody in Sample Diluent. Mix well and spin briefly.
15. Add 400 µl of the detection antibody to each well. Incubate at room temperature for 1-2 hours.

Note: Longer incubation time is preferable for higher signals.

16. Decant the samples from each well, and wash 5 times with 800 µl of 1x Wash Buffer I and then 2 times with 800 µl of 1x Wash Buffer II at room temperature with gentle shaking. Completely remove wash buffer between each wash step.

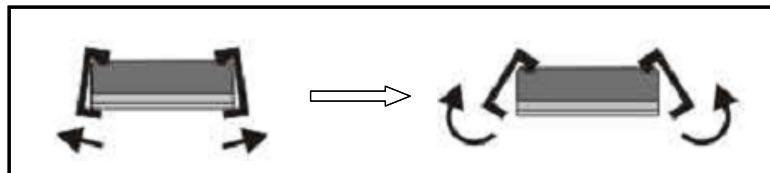
F. Incubation with Cy5 Equivalent Dye-Streptavidin

17. Prepare 1X Dye-conjugated Streptavidin:
 - a) Briefly spin down the Cy5 equivalent dye-conjugated streptavidin tube (Item I) immediately before use.
 - b) Add 850 µl of Sample Diluent to the tube to prepare a 1X Cy5-Conjugated Streptavidin solution. Mix gently (do not store the stock solution for later use).
18. Add 400 µl of 1X Cy5 equivalent dye-conjugated streptavidin to each well. Cover the incubation chamber with the plastic adhesive strips (Item J) and cover the slide with aluminum foil to avoid exposure to light or incubate in dark room.
19. Incubate the slide with Cy5-Conjugated Streptavidin at RT for 1 hour with gentle rocking or shaking.
20. Decant the samples from each well, and wash 5 times with 800 µl of 1x Wash Buffer I at room temperature with gentle shaking. Completely remove wash buffer in each wash step.

G. Fluorescence Detection

21. Disassemble the slide assembly by pushing clips outward from the slide side, as shown below. Carefully remove the slide from the gasket.

Note: Be careful not to touch the surface of the array.



22. Gently place the slide in the slide Washer/Dryer (a 4-slide holder/centrifuge tube, Item M), add enough 1x Wash Buffer I (about 30 ml) to cover the whole slide, and then gently shake at room temperature for 15 minutes. Decant Wash Buffer I. Wash with 1x Wash Buffer II (about 30 ml) and gently shake at room temperature for 5 minutes.
23. Finally, wash the glass slide with 30 mL of de-ionized or distilled water for 5 min.
24. Remove water droplets completely by gently applying suction with a pipette. Do not touch the sub-array areas, only the sides of the slide.

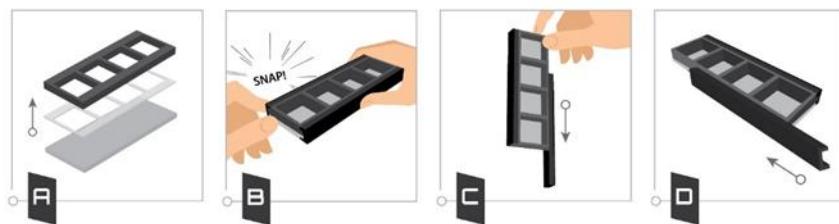
Make sure the finished glass slide is completely dry before scanning or storage.

25. Imaging: The signals can be visualized through use of a laser scanner equipped with a Cy5 wavelength such as Axon GenePix. Make sure that the signal from the spot containing the highest concentration receives the highest possible reading, yet remains unsaturated.

Note: Unlike most Cy5 fluors, the Streptavidin-Conjugated Fluor used in this kit is very stable at RT and resistant to photobleaching on completed glass slides. However, please protect glass slides from temperatures above RT and store them in the dark. Do not expose glass slide to strong light, such as sunlight or UV lamp.

Note: If you need to repeat any of the incubations after finishing the experiment, you must first re-assemble the glass slide into the incubation chamber by following step as shown in the figures below. To avoid breaking the printed glass slide, you may first want to practice assembling the device with a blank glass slide.

1. Apply slide to incubation chamber facing upward (image A).
2. Gently snap one edge of a snap-on side (image B).
3. Gently press other of side against lab bench and push in lengthwise direction (image C).
4. Repeat with the other side (image D)



H. Data Analysis

Data extraction can be done with most of the microarray analysis software (GenePix, ScanArray Express, ArrayVision, or MicroVigene).

Glycan 300 Map

V. Glycan Array Map

The glycans corresponding with the position IDs below can be found in the Appendix. Glycan structures can be downloaded here: http://www.raybiotech.com/files/Tech-Support/RayBiotech_Glycan-300_structures.pdf

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	PO1	PO1	PO1	PO1	PO2	PO2	NEG	NEG	G0001	G0001	G0001	G0002	G0002	G0003	G0003	G0004	G0004	G0005	G0005	G0005	G0006	G0006	G0006	G0006	G0006		
2	G0007	G0007	G0007	G0008	G0008	G0008	G0009	G0009	G0009	G0010	G0010	G0010	G0011	G0011	G0012	G0012	G0013	G0013	G0014	G0014	G0014	G0015	G0015	G0015	G0015		
3	G0016	G0016	G0016	G0017	G0017	G0017	G0018	G0018	G0019	G0019	G0020	G0020	G0021	G0021	G0022	G0022	G0023	G0023	G0024								
4	G0025	G0025	G0025	G0026	G0026	G0026	G0027	G0027	G0028	G0028	G0029	G0029	G0030	G0030	G0031	G0031	G0032	G0032	G0033								
5	G0034	G0034	G0034	G0035	G0035	G0035	G0036	G0036	G0037	G0037	G0038	G0038	G0039	G0039	G0040	G0040	G0041	G0041	G0042								
6	G0043	G0043	G0043	G0044	G0044	G0044	G0045	G0045	G0046	G0046	G0047	G0047	G0048	G0048	G0049	G0049	G0050	G0050	G0050	G0051							
7	G0052	G0052	G0052	G0053	G0053	G0053	G0054	G0054	G0055	G0055	G0056	G0056	G0057	G0057	G0058	G0058	G0059	G0059	G0059	G0060							
8	G0061	G0061	G0062	G0062	G0063	G0063	G0064	G0064	G0065	G0065	G0066	G0066	G0067	G0067	G0068	G0068	G0069										
9	G0070	G0070	G0071	G0071	G0072	G0072	G0073	G0073	G0074	G0074	G0075	G0075	G0076	G0076	G0077	G0077	G0078										
10	G0079	G0079	G0080	G0080	G0081	G0081	G0082	G0082	G0083	G0083	G0084	G0084	G0085	G0085	G0086	G0086	G0087										
11	G0088	G0088	G0088	G0089	G0089	G0089	G0090	G0090	G0091	G0091	G0092	G0092	G0093	G0093	G0094	G0094	G0095	G0095	G0095	G0096	G0096	G0096	G0096	G0096	G0096		
12	G0097	G0097	G0097	G0098	G0098	G0098	G0099	G0099	G0100	G0100	G0110	G0110	G0110	G0110	G0111	G0111	G0112	G0112	G0113	G0113	G0114	G0114	G0114	G0114	G0114		
13	N015	N015	N020	N020	N021	N021	N022	N022	N023	N023	N024	N024	N025	N025	N026												
14	N023G	N023G	N023G	N025G	N025G	N025G	N030	N030	N210	N210	N040	N040	N041	N041	N042	N042	N043	N043	N044								
15	N045	N045	N050	N050	N051	N051	N052	N052	N053	N053	N054	N054	N055														
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18	TE021	TE021	TE022	TE022	TE023	TE023	TE024	TE024	TE025	TE025	TE026	TE026	TE027	TE027	TE028	TE028	TE029										
19	TE030	TE030	TE031	TE031	TE032	TE032	TE033	TE033	TE034	TE034	TE035	TE035	TE036	TE036	TE037	TE037	TE037	TE038									
20	TE039	TE039	TE040	TE040	TE040	TE041	TE041	TE042	TE042	TE043	TE043	TE044	TE044	TE045	TE045	TE046	TE046	TE046	TE047								
21	TE048	TE048	TE049	TE049	TE049	TE050																					
22	H0201	H0201	H0202	H0202	H0202	H0203	H0203	H0204	H0204	H0204	H0205	H0205	H0205	H0207	H0207	H0208	H0208	H0208	H0209	H0209	H0209	H0210	H0210	H0210	H0210		
23	H0300	H0300	H0301	H0301	H0303	H0303	H0304	H0304	H0305	H0305	H0306	H0307	H0307	H0404	H0404	H0404	H0404	H0404	H0404								
24	H0403	H0403	H0404	H0404	H0404	H0405	H0405	H0504	H0504	H0505																	
25	H0606	H0606	H0608	H0608	H0609	H0609	H0610	H0610	H0700	H0700	H0707																
26	L1002	L1002	L1002	L1003	L1003	L1003	L1011	L1011	L1012	L1012	L1013	L1013	L1013	L1013	L1021	L1021	L1022	L1022	L1023	L1023	L1201	L1201	L1201	L1201	L1201		
27	L1202	L1202	L1202	L1203	L1203	L1204	L1204	L1205	L1205	L1206	L1206	L1206	L1207	L1207	L1209	L1209	L1211	L1211	L1212								
28	L1213	L1213	L1213	L1214	L1214	L1214	L1215	L1215	L1216	L1216	L1221	L1221	L1222	L1222	L1225	L1225	L1226	L1226	L1226	L1226	L2000	L2000	L2000	L2000	L2000		
29	L2100	L2100	L2101	L2101	L2101	L2102	L2102	L2111	L2111	L2112	L2112	L2113	L2113	L2113	L2113	L2121	L2121	L2122									
30	L2104	L2104	L2131	L2131	L2132	L2132	L2132	L2133	L2133	L2191	L2191	L2192	L2192	L2192	L2192	L2200	L2200	L2300	L2300	L2301	L2301	L2301	L2301	L2301	L2301		
31	L2302	L2302	L2311	L2311	L2312	L2312	L2303	L2303	L2304	L2304	L2391	L2391	L2392	L2392	L2900	L2900	L2911										
32	L2912	L2912	L2913	L2913	L3100	L3100	L3101	L3101	L3102	L3102	L3111	L3111	L3112	L3112	L3113	L3113	L3103										
33	L3200	L3200	L3201	L3201	L3202	L3202	L3202	L3202	L3202	L3202	L3211	L3211	L3212	L3212	L3213	L3213	NEG	NEG	POS2	POS2	POS1	POS1	POS1	POS1	POS1		
34	L3200	L3200	L3201																								

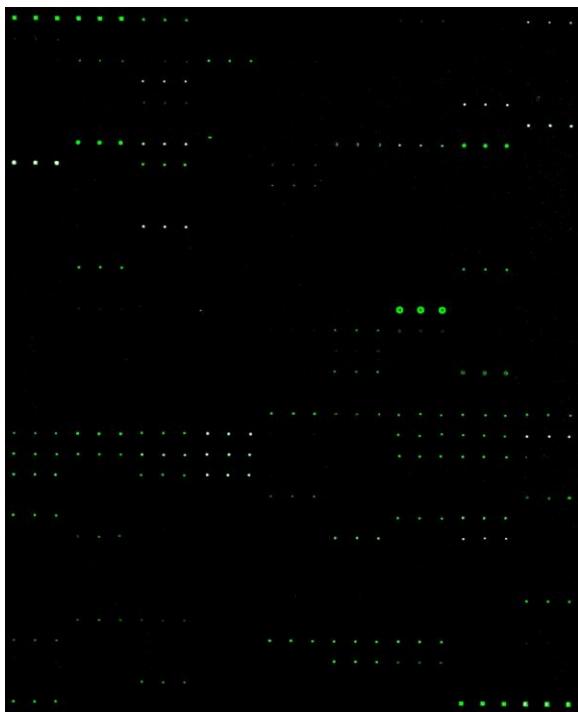
VI. Interpretation of Results and Typical Examples:

A. Explanation of Controls Spots

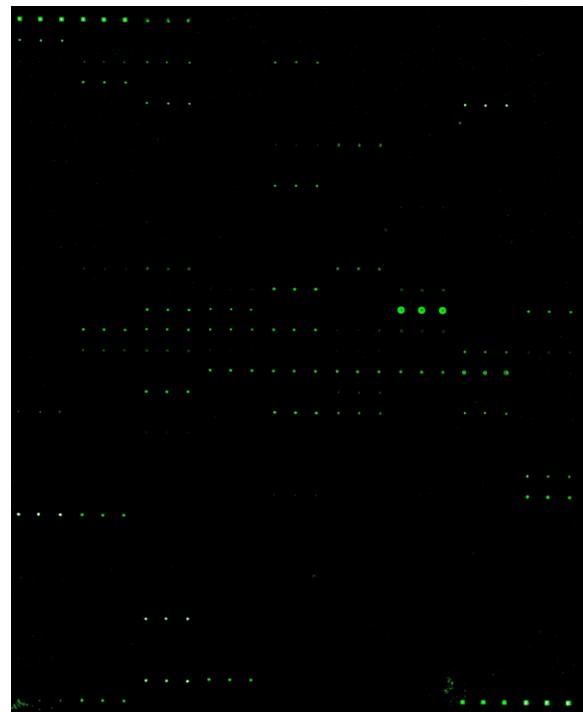
- 1) **Positive Control spots (POS1, POS2, POS3)** are standardized amounts of biotinylated protein printed directly onto the array. All other variables being equal, the Positive Control intensities will be the same for each sub-array. This allows for normalization based upon the relative fluorescence signal responses to a known control, much as “housekeeping” genes or proteins are used to normalize results in PCR or Western blots, respectively.
- 2) **Negative Control (NEG)** spots contain a buffer only (used to dilute glycans printed on the array). Their signal intensities represent non-specific binding of Biotin-conjugated anti-Cytokines and/or the Cy5-Conjugated Streptavidin. Negative control signal intensities are usually very close to background signals in each sub-array.

B. Typical Results Obtained with RayBio® Glycan Array 300

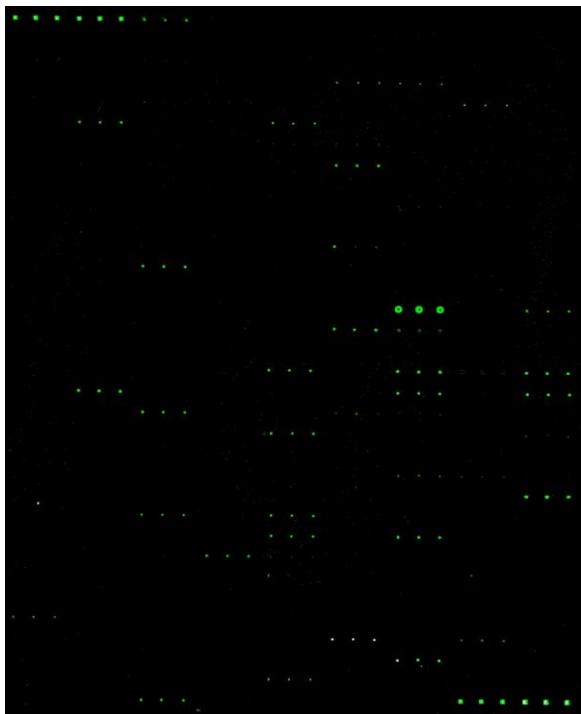
The following figures show the RayBio® Glycan Array 300 probed with biotin-labeled lectin mixtures and a serum sample. The images were captured using an Axon GenePix laser scanner. The strong signals in the upper left and lower right corners of each array are Positive Controls, which can be used to identify the orientation and help normalize the results between arrays. Interaction with different lectin mixtures, which have the ability to bind to different glycans with corresponding moieties such as αGal, αFuc, GlcNAc et al., the Glycan-300 array displays strong signals with different patterns. When incubated with a biotinylated serum sample, glycans in the array showed strong binding activity, indicating that the serum sample contains spectrometric proteins with binding activity to different glycans.



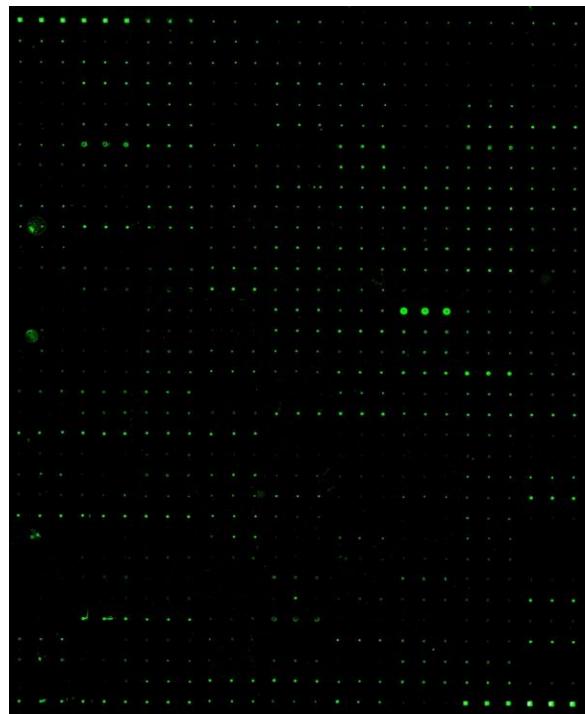
Biotinylated lectin mixture:
BSL I, WGA, UEA I, and Con A
(0.04 µg/mL)



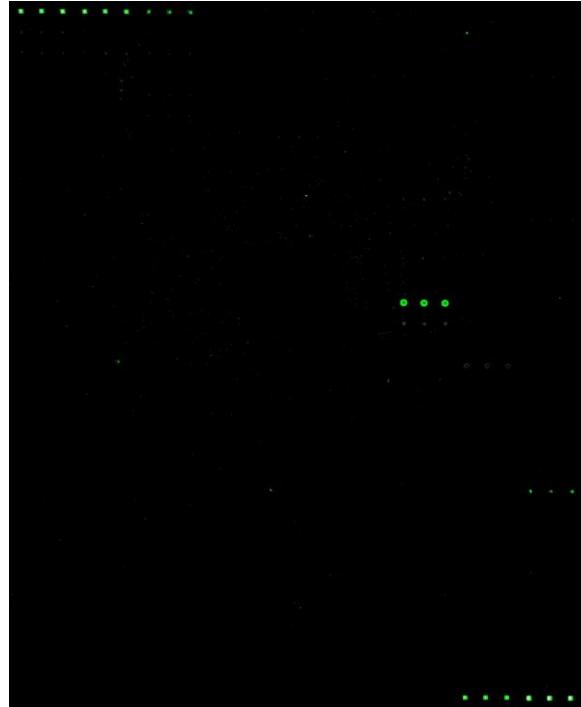
Biotinylated lectin mixture:
SBA, PHA-E and PSA (0.4
µg/mL)



Biotinylated lectin mixture:
DBA, PNA and RCA-I (0.4
µg/mL)



Biotinylated serum sample



Background control

If scanned using optimal settings, 2 distinct signal intensities will be seen: POS1>POS2. If these signals are of similar intensity, try increasing or decreasing laser power and/or signal gain settings.

C. Background Subtraction:

Once you have obtained fluorescence intensity data, you should subtract the background and normalize to the Positive Control signals before proceeding to analysis.

Most laser fluorescence scanner software has an option to automatically measure the local background around each spot. For best results, we recommend comparing signal intensities representing the MEDIAN background signals minus local background. If your resulting fluorescence signal intensity reports do not include these values (e.g., a column labeled as "MED532-B532"), you may need to subtract the background manually or change the default settings on your scanner's data report menu.

D. Normalization of Array Data:

To normalize signal intensity data, one sub-array is defined as "reference" to which the other arrays are normalized. This choice is arbitrary. For example, in our Analysis Tool Software (described below), the array represented by data entered in the left-most column each worksheet is the default "reference array."

You can calculate the normalized values as follows:

$$X(Ny) = X(y) * P1/P(y)$$

Where:

P1 = mean signal intensity of POS spots on reference array

P(y) = mean signal intensity of POS spots on Array "y"

X(y) = mean signal intensity for spot "X" on Array "y"

X(Ny) = normalized signal intensity for spot "X" on Array "y"

The RayBio® Analysis Tool software is available for use with data obtained using RayBio® Glycan Array 300. You can copy and paste your signal intensity data (with and without background) into the Analysis Tool, and it will automatically normalize signal intensities to the Positive Controls.

To order the Analysis Tool, please contact us at +1-770-729-2992 or info@raybiotech.com for more information.

E. Threshold of significant difference in samples:

After subtracting background signals and normalization to Positive Controls, comparison of signal intensities between and among array images can be used to determine relative differences between samples or groups.

Any ≥ 1.5 -fold increase or ≤ 0.65 -fold decrease in signal intensity for a single analyte between samples or groups may be considered a measurable and significant difference in expression, provided that both sets of signals are well above background (Mean background + 2 standard deviations, accuracy $\approx 95\%$).

VII. Troubleshooting Guide

Problem	Cause	Recommendation
Weak Signal	Inadequate detection	Increase laser power and PMT parameters
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Short incubation time	Ensure sufficient incubation time or change sample incubation step to overnight
	Too low glycan concentration in sample	Reduce amount of dilution or concentrate sample
	Improper storage of kit	Store kit as suggested temperature; Don't freeze/thaw the slide
Uneven Signal	Bubble formed during incubation	Handle and pipette solutions more gently; De-gas solutions prior to use
	Arrays are not completely covered by reagent	Prepare more reagent and completely cover arrays with solution
	Reagent evaporation	Cover the incubation chamber with adhesive film during incubation
General	Cross-contamination from neighboring wells	Avoid overflowing wash buffer
	Comet tail formation	Air dry the slide for at least 1 hour before usage
	Inadequate detection	Increase laser power that the highest concentration for each lectin receives the highest possible reading yet remains unsaturated
High Background	Overexposure	Lower the laser power
	Dark spots	Completely remove wash buffer in each wash step
	Insufficient wash	Increase wash time and use more wash buffer
	Dust	Minimize dust in work environment before starting experiment
	Slide is allowed to dry out	Take additional precautions to prevent slides from drying out during experiment

Appendix

Glycan Names and Position ID – Download this List Here: <http://www.raybiotech.com/files/Tech-Support/Glycan-300-list.pdf>

Glycans

- G0001: β -Glc-Sp
- G0002: β -Gal-Sp
- G0003: α -Man-Sp
- G0004: α -Fuc-Sp
- G0005: α -Rha-Sp
- G0006: β -GlcNAc-Sp
- G0007: β -GalNAc-Sp
- G0008: Tobramycin
- G0009: Gal- β -1,3-GlcNAc- β -Sp
- G0010: Gal- α -1,3-Gal- β -1,3-GlcNAc- β -Sp
- G0011: Neu5Ac- α -2,3-Gal- β -1,3-GlcNAc- β -Sp
- G0012: Neu5Ac- α -2,6-Gal- β -1,3-GlcNAc- β -Sp
- G0013: Neu5Gc- α -2,3-Gal- β -1,3-GlcNAc- β -Sp
- G0014: Neu5Gc- α -2,6-Gal- β -1,3-GlcNAc- β -Sp
- G0015: Gal- β -1,3-(Fuc- α -1,4)-GlcNAc- β - [Lewis A] -Sp
- G0016: Gal- β -1,4-Glc- β -Sp
- G0017: Gal- α -1,3-Gal- β -1,4-Glc- β -Sp
- G0018: Gal- α -1,4-Gal- β -1,4-Glc- β -Sp
- G0019: GlcNAc- β -1,3-Gal- β -1,4-Glc- β -Sp
- G0020: GalNAc- β -1,3-Gal- β -1,4-Glc- β -Sp
- G0021: Neu5Ac- α -2,3-Gal- β -1,4-Glc- β -Sp
- G0022: Neu5Ac- α -2,6-Gal- β -1,4-Glc- β -Sp
- G0023: Neu5Gc- α -2,3-Gal- β -1,4-Glc- β -Sp
- G0024: Neu5Gc- α -2,6-Gal- β -1,4-Glc- β -Sp
- G0025: Gal- β -1,4-(Fuc- α -1,3)-Glc- β -Sp
- G0026: GalNAc- β -1,3-Gal- α -1,4-Gal- β -1,4-Glc- β -Sp
- G0027: GlcNAc- β -1,6-GlcNAc- β -Sp
- G0028: 4-P-GlcNAc-b-1,4-Man-b-Sp
- G0029: Glc- α -1,2-Gal- α -1,3-Glc- α -Sp
- G0030: Gal- β -1,3-GalNAc- α -Sp
- G0031: Gal- β -1,4-GlcNAc- β -Sp
- G0032: Gal- β -1,4 -(Fuc- α -1,3)-GlcNAc- β - [Lewis X] -Sp
- G0033: Neu5Ac- α -2,3-Gal- β -1,4-(Fuc- α -1,3)-GlcNAc- β - [Sialyl Lewis X]-Sp
- G0034: Neu5Ac- α -2,3-Gal- β -1,3 -(Fuc- α -1,4)-GlcNAc- β - [Sialyl Lewis A]-Sp
- G0035: Neu5Gc- α -2,3-Gal- β -1,3-(Fuc- α -1,4)-GlcNAc- β - [Sialyl Lewis A]-Sp
- G0036: Gal- α -1,4-Gal- β -1,3-GlcNAc- β -Sp
- G0037: Gal- β -1,4-GlcNAc- β -1,3-Gal- β -1,4-Glc- β - [LNnT]-Sp
- G0038: GlcA- β -1,4-GlcNAc- α -1,4-GlcA- β -Sp
- G0039: GlcNAc- β -1,6-(Gal- β -1,3)-GalNAc- α -O-Ser-Sp4
- G0040: Neu5Ac- α -2,3Gal- β -1,4-(6S)GlcNAc- β -Sp
- G0041: GalNAc- β -1,4-GlcNAc- β -Sp2
- G0042: Neu5Ac- α -2,8-Neu5Ac- α -2,3-Gal β -1,4-Glc- β -Sp
- G0043: Neu5Gc- α -2,8-Neu5Ac- α -2,3-Gal- β -1,4-Glc- β -Sp
- G0044: GalNAc- α -1,3-(Fuc- α -1,2)-Gal- β -1,4-Glc- β - [Blood A antigen tetrose]-Sp1
- G0045: GlcNAc- β -1,2-Man- α -Sp
- G0046: Neu5Ac- α -2,3-Gal- β -Sp1
- G0047: Gal- β -1,3 -GalNAc- β -1,3-Gal- β -Sp1
- G0048: Gal- α -1,2-Gal- α -Sp
- G0049: Gal- β -1,4-(Fuc- α -1,3)-GlcNAc- β -1,3-Gal- β -Sp1
- G0050: Neu5Ac- α -2,3-Gal- β -1,4-(Fuc- α -1,3)-Glc- β - [3-Sialyl-3-fucosyllactose/ F-SL]-Sp1
- G0051: GlcNAc- β -1,4-GlcNAc- β -Sp1
- G0052: β -D-GlcA-Sp
- G0053: Gal- β -1,4-(6S)GINAc- β -Sp
- G0054: GlcNAc- α -1,3-(Glc- α -1,2-Glc- α -1,2)-Gal- α -1,3-Glc- α -Sp
- G0055: Gal- β -1,3-GalNAc- β -1,4-(Neu5Gc- α -2,3)-Gal- β -1,4-Glc- β -Sp1
- G0056: Sisomicin Sulfate
- G0057: GalNAc- α -1,3-(Fuc- α -1,2)-Gal- β - [Blood A antigen trisaccharide]-Sp1
- G0058: Fuc- α -1,2-Gal- β -1,4-GlcNAc- β - [Blood H antigen trisaccharide]-Sp1
- G0059: Gal- α -1,3-(Fuc- α -1,2)-Gal- β - [Blood B antigen trisaccharide]-Sp1
- G0060: Fuc- α -1,2-Gal- β -1,3-GlcNAc- β -1,3-Gal- β -1,4-Glc- β - [LNFP I]-Sp1
- G0061: Fuc- α -1,2-Gal- β -1,4-Glc- β - [Blood H antigen trisaccharide]-Sp1
- G0062: Gal- α -1,3-(Fuc- α -1,2)-Gal- β -1,4-Glc- β - [Blood B antigen tetrasaccharide]-Sp1

- G0063: (Fuc- α -1,2)-Gal- β -1,4-(Fuc- α -1,3)-GlcNAc- β - [Lewis Y]-Sp1
- G0064: (Fuc- α -1,2)-Gal- β -1,3-(Fuc- α -1,4)-GlcNAc- β - [Lewis B]-Sp1
- G0065: Gal- β -1,3-(Fuc- α -1,4)-GlcNAc- β -1,3-Gal- β -1,4-(Fuc- α -1,4)-Glc- β - [Lewis A]-Sp1
- G0066: Gal- β -1,3-GalNAc- β -Sp1
- G0067: Gal- β -1,3-(Neu5Ac- α -2,6)-GalNAc- β -Sp
- G0068: Neu5Ac- α -2,6-Gal- β -1,3-GalNAc- β -Sp
- G0069: Neu5Ac- α -2,6-Gal- β -1,3-(Neu5Ac- α -2,6)-GalNAc- β -Sp
- G0070: Neu5Ac- α -2,3-Gal- β -1,3-(Neu5Ac- α -2,6)-GalNAc- β -Sp
- G0071: Neu5Ac- α -2,6-(Neu5Ac- α -2,3)-Gal- β -1,3-GalNAc- β -Sp
- G0072: GalNAc- β -1,4-(Neu5Ac- α -2,3)-Gal- β -1,4-Glc- β - [GM2]-Sp
- G0073: GalNAc- β -1,4-(Neu5Ac- α -2,8)-Neu5Ac- α -2,3)-Gal- β -1,4-Glc- β - [GD2]-Sp
- G0074: Gal- α -1,4-Gal- β -1,4-GlcNAc- β -Sp1
- G0075: β -D-Rha-Sp
- G0076: Glc- α -1,4-Glc- β -Sp1
- G0077: Glc- α -1,6-Glc- α -1,4-Glc- β -Sp1
- G0078: Maltotriose- β -Sp1
- G0079: Glc- α -1,6-Glc- α -1,6-Glc- β -Sp1
- G0080: Maltotetraose- β -Sp1
- G0081: GlcNAc- α -1,4-GlcA- β -1,4-GlcNAc- α 1,4-GlcA- β -Sp
- G0082: Maltohexaose- β -Sp1
- G0083: Maltoheptaose- β -Sp1
- G0084: Acarbose- β -Sp1
- G0085: D-pentamannuronic acid- β -Sp1
- G0086: L-pentaguluronic acid- β -Sp1
- G0087: D-cellose- β -Sp1
- G0088: Gal- α -1,3-Gal- β -Sp1
- G0089: β -1,4-Xylotetrose-Sp1
- G0090: Chitin-trisaccharide-Sp1
- G0091: KDN- α -2,8-Neu5Ac- α -2,3-Gal- β -1,4-Glc- β -Sp
- G0092: Neu5Ac- α -2,8-Neu5Gc- α -2,3-Gal- β -1,4-Glc- β -Sp
- G0093: Neu5Ac- α -2,8-Neu5Ac- α -2,8-Neu5Ac- α -2,3-Gal- β -1,4-Glc- β -Sp3
- G0094: Neu5Ac- α -2,8-Neu5Ac- α -2,6-Gal- β -1,4-Glc-Sp5
- G0095: Gal- β -1,3-GalNAc- β -1,4-(Neu5Ac- α -2,3)-Gal- β -1,4-Glc- β -Sp1
- G0096: Gentamicin Sulfate
- G0097: Kanamycin sulfate
- G0098: Geneticin Disulfate Salt (G418)
- G0099: Neomycin trisulfate
- G0100: SGP

N-Glycans

- N010: Man- α -1,6-(Man- α -1,3-)Man- α -1,6-(GlcNAc- β -1,2-Man- α -1,3-)Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N011: Man- α -1,6-(Man- α -1,3-)Man- α -1,6-(Gal- β -1,4-GlcNAc- β -1,2-Man- α -1,3-)Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N012: Man- α -1,6-(Man- α -1,3-)Man- α -1,6-(Neu5Ac- α -2,3-Gal- β -1,4-GlcNAc- β -1,2-Man- α -1,3-) Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N013: Man- α -1,6-(Man- α -1,3-)Man- α -1,6-(Neu5Ac- α -2,6-Gal- β -1,4-GlcNAc- β -1,2-Man- α -1,3-) Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N014: Man- α -1,6-(Man- α -1,3-)Man- α -1,6-[Gal- β -1,4-(Fuc- α -1,3)-GlcNAc- β -1,2-Man- α -1,3]Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N015: Man- α -1,6-(Man- α -1,3-)Man- α -1,6-[Neu5Ac- α -2,3-Gal- β -1,4-(Fuc- α -1,3)-GlcNAc- β -1,2- Man- α -1,3]Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N020: GlcNAc- β -1,2-Man- α -1,3-Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N021: Gal- β -1,4-GlcNAc- β -1,2-Man- α -1,3-Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N022: Neu5Ac- α -2,3-Gal- β -1,4-GlcNAc- β -1,2-Man- α -1,3-Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N023: Neu5Ac- α -2,6-Gal- β -1,4-GlcNAc- β -1,2-Man- α -1,3-Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N024: Gal- β -1,4-(Fuc- α -1,3)-GlcNAc- β -1,2-Man- α -1,3-Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N025: Neu5Ac- α -2,3-Gal- β -1,4-(Fuc- α -1,3)-GlcNAc- β -1,2-Man- α -1,3-Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N022G: Neu5Gc- α -2,3-Gal- β -1,4-GlcNAc- β -1,2-Man- α -1,3-Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N023G: Neu5Gc- α -2,6-Gal- β -1,4-GlcNAc- β -1,2-Man- α -1,3-Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N025G: Neu5Gc- α -2,3-Gal- β -1,4-(Fuc- α -1,3)-GlcNAc- β -1,2-Man- α -1,3-Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N026: Gal- α -1,3-Gal- β -1,4-GlcNAc- β -1,2-Man- α -1,3-Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N030: Man- α -1,6-(GlcNAc- β -1,2-Man- α -1,3-)Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N040: GlcNAc- β -1,2-Man- α -1,6-Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N041: Gal- β -1,4-GlcNAc- β -1,2-Man- α -1,6-Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N042: Neu5Ac- α -2,3-Gal- β -1,4-GlcNAc- β -1,2-Man- α -1,6-Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N043: Neu5Ac- α -2,6-Gal- β -1,4-GlcNAc- β -1,2-Man- α -1,6-Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N044: Gal- β -1,4-(Fuc- α -1,3)-GlcNAc- β -1,2-Man- α -1,6-Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N045: Neu5Ac- α -2,3-Gal- β -1,4-(Fuc- α -1,3)-GlcNAc- β -1,2-Man- α -1,6-Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N050: GlcNAc- β -1,2-Man- α -1,6-(Man- α -1,3-)Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N051: Gal- β -1,4-GlcNAc- β -1,2-Man- α -1,6-(Man- α -1,3-)Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N052: Neu5Ac- α -2,3-Gal- β -1,4-GlcNAc- β -1,2-Man- α -1,6-(Man- α -1,3-)Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N053: Neu5Ac- α -2,6-Gal- β -1,4-GlcNAc- β -1,2-Man- α -1,6-(Man- α -1,3-)Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp
- N054: Gal- β -1,4-(Fuc- α -1,3)-GlcNAc- β -1,2-Man- α -1,6-(Man- α -1,3-)Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N055: Neu5Ac- α -2,3-Gal- β -1,4-(Fuc- α -1,3)-GlcNAc- β -1,2-Man- α -1,6-(Man- α -1,3-)Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5
- N210: GlcNAc- β -1,2-Man- α -1,6-[GlcNAc(3Ac)- β -1,2-Man- α -1,3-Man- β -1,4-GlcNAc- β -1,4-GlcNAc-Sp5

Glycolipid Glycans

- L1001: Neu5Ac- α -2,3-Gal- β -1,4-Glc-Sp5

Human Milk Oligosaccharides

- H0100: GlcNAc- β -1,3-(GlcNAc- β -1,6-)Gal- β -1,4-Glc-Sp5
 - H0101: Gal- β -1,4-GlcNAc- β -1,3-(Gal- β -1,4-GlcNAc- β -1,6-)Gal- β -1,4-Glc-Sp5

RayBio® Glycan Array 300

Tandem epitopes

Note:

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