RayBiotech Lectin Array 95

--Detect glycan profiles using 95 lectins

User Manual
July 11th, 2022

Cat # GA-Lectin-95

RayBiotech, Inc.

We Provide You With Excellent
Protein Array Systems and Service

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Lectins printed on slides (95)	AAA, AAL, ABA, ABL, ACG, ACL, AMA, ASA, BanLec, BC2L-A, BC2LCN, BPA, CA, CAA, Calsepa, CGL2, CNL, Con A, CPA, CSA, DBA, Discoidin I, Discoidin II, DSA, ECA, EEL, F17AG, Gal1, Gal1-S, Gal2, Gal3, Gal3C-S, Gal7-S, Gal9, GHA, GNA, GRFT, GS-I, GS-II, HAA, HHA, HMA, IRA, Jacalin, LAL, LBA, LCA, LEA, Lentil, Lotus, LPA, LSL-N, MAA, Malectin, MNA-G, MNA-M, MOA, MPL, NPA, Orysata, PA-IIL, PA-IL, PALa, PHA-E, PHA-L, PHA-P, PNA, PPL, PSA, PSL1a, PTL-1, PTL-2, PWA, RCA-120, RCA-60, RPA, RS-Fuc, SAMB, SBA, SHA, SJA, SNA-I, SNA-II, SSA, STL, TL, UDA, UEA-I, UEA-II, VFA, VRA, VVA, VVA-M, WFA, WGA
Format	One standard glass slide is spotted with 12 wells of identical lectin sub-arrays. Each lectin is printed in duplicate on every sub-array
Detection Method	Fluorescence with laser scanner: Cy3 equivalent dye
Sample Volume	50 – 100 μl per array
Reproducibility	CV <20%
Assay duration	6 hrs

TABLE OF CONTENTS

I. Overview

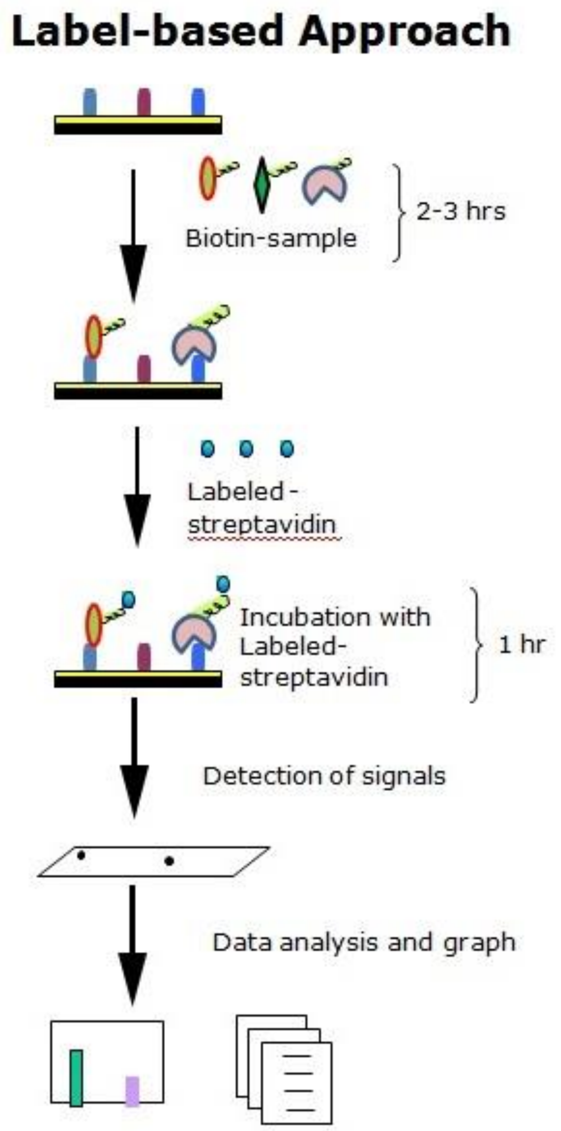
A. Introduction

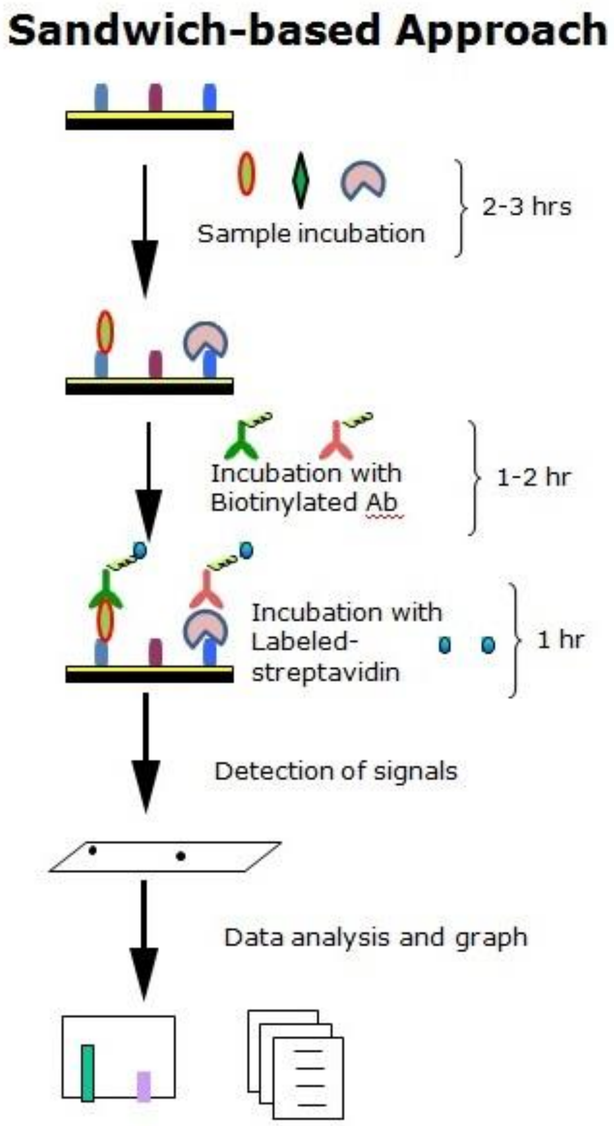
Glycocalyx, literally meaning ‘sugar coat’, is an extracellular polymeric coating surrounding many prokaryotic and eukaryotic cells consisting of glycoproteins, glycolipids, proteoglycans and glycosaminoglycans. The constituents of the glycocalyx play an important role in the process of cell signaling,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

RayBiotech, pioneered the development of antibody arrays, which are now widely applied in the research community with hundreds of peer reviewed publications such as in Cell and Nature. Taking advantage of advancements in microarray technology developed for antibody arrays, we recently developed lectin arrays to help researchers: 1) identify and profile the glycans in their samples; 2) determine whether their biomarker of interest has glycan moieties, and; 3) find specific glycan binding ligands in biological samples.

Lectins are glycan-binding proteins which have been purified from trees, beans and some fruits. They are highly specific for a given glycan based on their sequence and the different sugar unit structures the glycan contains. Based on the previously launched Lectin Array 40 and Lectin Array 70, RayBiotech now introduces the Lectin Array 95, which is currently the largest commercially available lectin array. The Lectin Array 95 consists of a standard glass slide spotted with 12 identical lectin arrays (1 per well), each containing 95 unique lectins spotted in duplicate, plus positive controls. The slide comes with a 16-well removable gasket which allows for the processing of 12 samples using one slide. Four slides can be nested into a tray, which matches a standard microplate and allows for the automated robotic high-throughput processing of 32 arrays simultaneously. The RayBio® Lectin Array 95 array provides a powerful new tool for glycosylation determination, drug discovery, and biomarker development, all while requiring limited sample volumes.

B. How it Works





II. Materials Provided

Upon receipt, all components of the RayBiotech Lectin Array 95 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

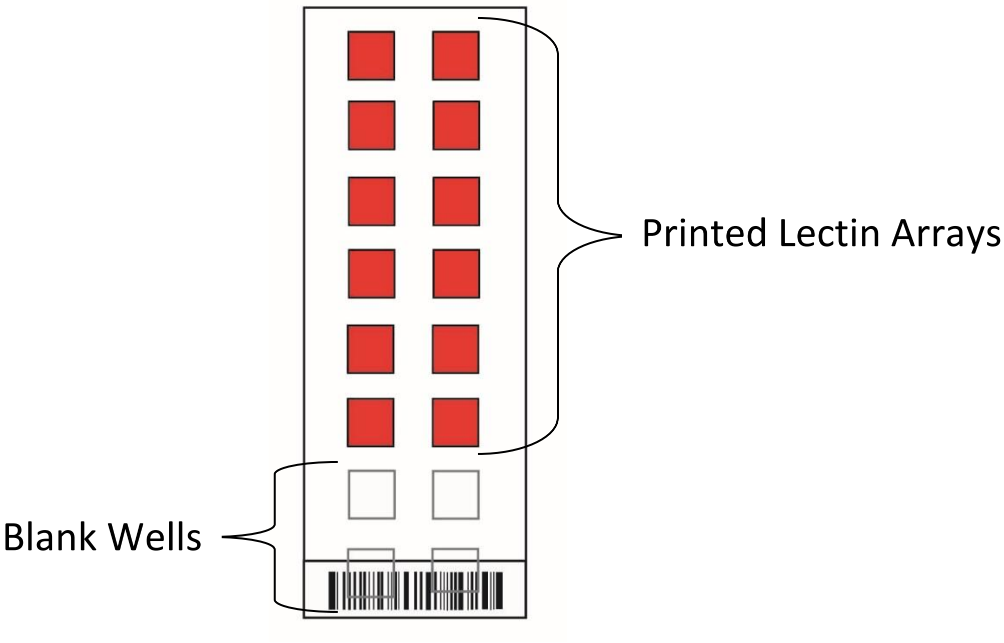
Item	**Description**	1-Slide kit	2-Slide kit	4-Slide kit
A	Dialysis Vials and Floating Dialysis Rack	24 vials/2 racks	48 vials/4 racks	96 vials/8 racks
B	Labeling Reagent	4	8	16
D	Stop Solution	1	2	4
E	Lectin Array Glass Slide Assembly	1	2	4
F	Sample Diluent	1	1	2
G	20X Wash Buffer I	1	1	2
H	20X Wash Buffer II	1	1	2
I	Cy3 equivalent dye-conjugated Streptavidin	1	2	4
J	Adhesive device sealer	2	4	8
K	Labeling Buffer	1	2	4
M	Slide Washer/Dryer	1	1	2
N	Manual	1	1	1

Additional Materials Required

- Detection antibodies of interest (For sandwich-based method only)
- Orbital shaker
- Laser scanner for fluorescence detection
- Aluminum foil
- 1.5ml Polypropylene microcentrifuge tubes
- KCl, NaCl, KH_2PO_4 and Na_2HPO_4 (For label-based method only)
- Plastic or glass containers, beaker, stir plate and stir bar
- Pipettors, pipette tips, ddH_2O and other common lab consumables

III. Glass Slide Layout

The Lectin Glass Slide includes 12 wells that are printed with the lectin array, and 4 wells that have been left blank. Please use the diagram below to ensure loading of samples in the correct wells.



IV. General Considerations

A. Label-Based vs. Sandwich-Based Method

The RayBiotech Lectin Array 95 Kit can be used with either a label-based method or as a sandwich-based method.

- The label-based method is used to biotinylate samples containing proteoglycans and glycoproteins for direct detection on the array via a Cy3 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 detection of glycocalyx elements (glycolipids, glycoproteins, etc.) captured on the array. The user will need to supply the labeled reporter antibodies specific for the glycocalyx elements 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

- Use serum-free conditioned media if possible.
- If serum-containing conditioned media is required, it is highly recommended that complete medium be used as a control since many types of sera contain glycocalyx elements.
- We recommend the following parameters for your samples:
  - 50 to 200 µl of original or diluted serum, plasma, cell culture media, or other body fluid, or 50-100 µl of cell or tissue lysates with 1-2 mg/ml total protein concentration.

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

C. Handling Glass Slides

- Do not touch the surface of the slides, as the microarray slides are very sensitive. Hold the slides by the edges only.
- Handle all buffers and slides with latex-free gloves.
- Handle the glass slide in a clean environment.
- 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.

D. Incubation

A. Completely cover array area with sample or buffer during incubation.

B. Avoid foaming during incubation steps.

C. Perform all incubation and wash steps under gentle rotation.

D. Cover the incubation chamber with the adhesive film during incubation to prevent evaporation, particularly when incubation is more than 2 hours or <70 μl of sample or reagent is used.

E. Several incubation steps such as step 6 (blocking), step 7 (sample incubation), step 10 (detection antibody incubation), or step 13 (Cy3 equivalent dye-streptavidin incubation) may be done overnight at 4^0C. Please make sure to cover the incubation chamber tightly to prevent evaporation.

V. Protocol

READ ENTIRE PROTOCOL BEFORE STARTING

A. Dialysis of Sample

*Note: For the Sandwich-based protocol start at **C. Drying the Glass Slide,** step 8, on page 10. Do not do steps 1-7.*

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 KH_2PO_4 and 1.15 g Na_2HPO_4 in 800 ml ddH_2O. Adjust pH=8.0 with 1M NaOH and adjust final volume to 1000 ml with ddH_2O.

2. Add each sample into a separate Dialysis Vial (Item A). Load 200 µl cell culture supernatant or 100 µl cell lysate 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 Vials into Floating Dialysis Rack.

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, Pierce, Catalog # 23227).

B. Biotin Labeling of 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 µl 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 µl dialyzed sample into a new tube. Add 36 µl 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 µl 1X Labeling Reagent to the tube of 180 µl dialyzed sample.

b. For labeling serum or plasma: Add 22 µl of 1X Labeling Reagent Solution into a new tube containing 35 µl dialyzed serum or plasma sample and 155 µl Labeling Buffer (Item K).

c. For labeling cell or tissue lysates: transfer 30 µg (for example, 15 µl of 2 mg/ml) cell or tissue lysates into a tube and add Labeling Buffer (Item K) for a total volume of 300 µl. Then add 3.3 µl 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.

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 µl 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

8. Take out the bag containing the glass slide from the box, and let the slide equilibrate to room temperature inside the sealed plastic bag for 20-30 minutes. Then, remove slide from the plastic bag; peel off the cover film, and let it air dry at room temperature for another 1-2 hours.

Note: Incomplete drying of slides before use may cause the formation of "comet tails".

D. Blocking and Incubation

9. Add 100µl Sample Diluent (Item F) into each well and incubate at room temperature for 30 min to block slides.

10. Immediately prior to sample incubation, spin biotin-labeled samples for 5 minutes at 10,000 rpm to remove any particulates or precipitates. Dilute samples with Sample Diluent. Recommended dilution of the biotin-labeled samples is 2-10 fold for cell culture supernatants, 20-100 fold for serum/plasma and 30-100 fold cell/tissue lysate, however, optimization is recommended to do for the best results.

11. Decant buffer from each well. Add 100µl of sample to each well. Incubate arrays at room temperature for 1-2 hours. *(Longer incubation time is preferable if higher signal intensity is desired)*

Note: We recommend using 50 to 100 µl of original or diluted serum, plasma, conditioned media, or other body fluid, or 50-500 µg/ml of protein for cell and tissue lysates. Cover the incubation chamber with adhesive film during incubation if less than 70 ul of sample or reagent is used.

Note: This step may be done overnight at 4^0C for highest intensities.

Note: For the Sandwich-based protocol, it's recommended to do optimization to determine appropriate dilution of non-biotinylated samples for incubation.

12. Wash:

    a. 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 ddH_2O 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 150 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 150 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". (Background signal is higher than that of the spot.)

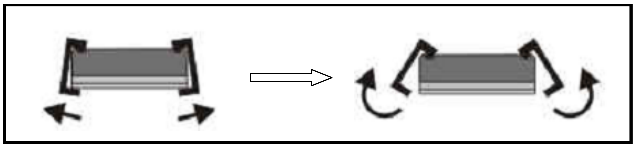
E. Incubation with Cy3 Equivalent Dye-Streptavidin

Note: For the Sandwich-based protocol, follow below steps after secondary antibody incubation if biotinylated secondary antibody is used. If fluorescence conjugated secondary antibody is used, skip steps 13-15 and continue from step 16 after incubation of secondary antibody. Appropriate dilution of secondary antibody should be determined before incubation.

13. Briefly spin down the Cy3 equivalent dye-conjugated streptavidin tube.
14. Add 1.4 ml of Sample Diluent to Cy3 equivalent dye-conjugated streptavidin tube. Mix gently.
15. Add 80 µl of Cy3 equivalent dye-conjugated streptavidin to each well. Cover the slide with aluminum foil to avoid exposure to light or incubate in dark room. Incubate at room temperature for 1 hour.
16. Decant the samples from each well, and wash 5 times with 150 µl of 1x Wash Buffer I at room temperature with gentle shaking. Completely remove wash buffer after each wash step.

F. Fluorescence Detection

17. Disassemble the slide assembly by pushing clips outward from the slide side. Carefully remove the slide from the gasket.



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

18. Place the slide in the slide Washer/Dryer (a 4-slide holder/centrifuge tube), 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) with gentle shaking at room temperature for 5 minutes.

19. Remove liquid droplets completely by one of the following ways:
    i. Put the glass slide into the Slide Washer/Dryer, and dry the glass slide by centrifuging at 1,000 rpm for 3 minutes without cap.
    ii. Or, dry the glass slide by a compressed N_2 stream.
    iii. Or gently apply suction with a pipette to remove water droplets. Do not touch the sub-array areas, only the sides of the slide.

20. Imaging: The signals can be visualized through use of a laser scanner equipped with a Cy3 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: If the signal intensity for different lectins vary greatly in the same array, we recommend using multiple scans, with a higher PMT for low signal lectins, and a low PMT for high signal lectins.

G. Data Analysis

21. Data extraction can be done using the GAL file that is specific for this array, along with the microarray software commonly available in most microarray laser scanners (GenePix, ScanArray Express, etc.). GAL files can be found on our website here. www.RayBiotech.com/Gal-Files.html.

H. Normalization of Array Data

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

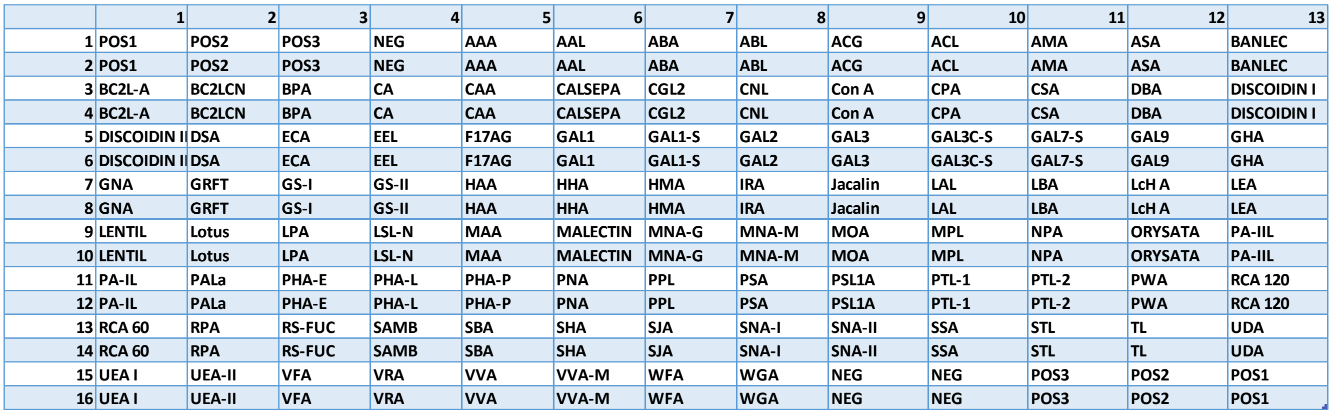
You can calculate the normalized values as follows:

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

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"

VI. Lectin Array 95 Map



	1	2	3	4	5	6	7	8	9	10	11	12	13
1	POS1	POS2	POS3	NEG	AAA	AAL	ABA	ABL	ACG	ACL	AMA	ASA	BANLEC
2	POS1	POS2	POS3	NEG	AAA	AAL	ABA	ABL	ACG	ACL	AMA	ASA	BANLEC
3	BC2L-A	BC2LCN	BPA	CA	CAA	CALSEPA	CGL2	CNL	Con A	CPA	CSA	DBA	DISCOIDIN I
4	BC2L-A	BC2LCN	BPA	CA	CAA	CALSEPA	CGL2	CNL	Con A	CPA	CSA	DBA	DISCOIDIN I
5	DISCOIDIN II	DSA	ECA	EEL	F17AG	GAL1	GAL1-S	GAL2	GAL3	GAL3C-S	GAL7-S	GAL9	GHA
6	DISCOIDIN II	DSA	ECA	EEL	F17AG	GAL1	GAL1-S	GAL2	GAL3	GAL3C-S	GAL7-S	GAL9	GHA
7	GNA	GRFT	GS-I	GS-II	HAA	HHA	HMA	IRA	Jacalin	LAL	LBA	LcH A	LEA
8	GNA	GRFT	GS-I	GS-II	HAA	HHA	HMA	IRA	Jacalin	LAL	LBA	LcH A	LEA
9	LENTIL	Lotus	LPA	LSL-N	MAA	MALECTIN	MNA-G	MNA-M	MOA	MPL	NPA	ORYSATA	PA-IIL
10	LENTIL	Lotus	LPA	LSL-N	MAA	MALECTIN	MNA-G	MNA-M	MOA	MPL	NPA	ORYSATA	PA-IIL
11	PA-IL	PALa	PHA-E	PHA-L	PHA-P	PNA	PPL	PSA	PSL1A	PTL-1	PTL-2	PWA	RCA 120
12	PA-IL	PALa	PHA-E	PHA-L	PHA-P	PNA	PPL	PSA	PSL1A	PTL-1	PTL-2	PWA	RCA 120
13	RCA 60	RPA	RS-FUC	SAMB	SBA	SHA	SJA	SNA-I	SNA-II	SSA	STL	TL	UDA
14	RCA 60	RPA	RS-FUC	SAMB	SBA	SHA	SJA	SNA-I	SNA-II	SSA	STL	TL	UDA
15	UEA I	UEA-II	VFA	VRA	VVA	VVA-M	WFA	WGA	NEG	NEG	POS3	POS2	POS1
16	UEA I	UEA-II	VFA	VRA	VVA	VVA-M	WFA	WGA	NEG	NEG	POS3	POS2	POS1

VII. Lectin Array 95 Key

	Lectins	**Abbreviation**	**Source**	**Carbonhydrate specificity**
1	*Anguilla anguilla*	AAA	*Anguilla anguilla* (Fresh Water Eel)	αFuc
2	*Aleuria aurantia*	AAL	*Aleuria aurantia* mushrooms	Fucα6GlcNAc
3	*Agaricus bisporus* lectin	ABA	Agaricus bisporus	Galactose (β1,3) N-Acetylgalactosamine
4	*Agaricus bisporus lectin*	ABL	*Agaricus bisporus* (White button mushroom)	galactose-β-1,3-N-acetylgalactosamine, galactose-β-1,3-N-acetylglucosamine
5	*Agrocybe cylindracea lectin*	ACG	E. coli expressed *Agrocybe cylindracea* galectin lectin	α2-3 Sialic Acid
6	*Amaranthus caudatus*	ACL, ACA	*Amaranthus caudatus* seeds	Galβ3GalNAc
7	*Arum maculatum lectin*	AMA	Arum maculatum (Lords and Ladies)	Mannose
8	*Allium sativum*	ASA	*Allium sativum* agglutinin (Garlic)	αMan
9	*Musa acuminata lectin*	BanLec	E. coli expressed *Musa acuminata*	containing α1,3-glycoside bond
10	*Burkholderia cenocepacia lectin*	BC2L-A	E. coli expressed *Burkholderia cenocepacia*	High-mannose
11	*Burkholderia cenocepacia lectin*	BC2LCN（AiLecS1)	E. coli expressed *Burkholderia cenocepacia*	Fucα1-2Galβ1-3GalNAc (H type 3), Fucα1-2Galβ1-3GlcNAc (H type 1)
12	*Bauhinia purpurea*	BPA, BLP	*Bauhinia purpurea alba* (Camel's Foot Tree)	Galβ3GalNAc
13	*Colchicum autumnale*	CA	Colchicum autumnale	Lactose > N-Acetylgalactosamine > Galactose
14	*Caragana arborescens*	CAA	Caragana arborescens (pea tree)	N-Acetylgalactosamine
15	*Calystegia sepium lectin*	Calsepa	E. coli expressed Calystegia sepium	High-mannose
16	*Coprinopsis cinerea lectin*	CGL2	E. coli expressed *Coprinopsis cinerea*	βGal, GalNAcα1-3Gal (Blood Group A), Galα1-3Gal (Blood Group B)
17	*Clitocybe nebularis lectin*	CNL	E. coli expressed *Clitocybe nebularis*	2]Galβ1-4GlcNAc (Blood Group A)
18	Coanavalin A	Con A	*Coanavalia ensformis* (Jack Beans) seeds	αMan, αGlc
19	Cicer arietinum lectin	CPA	Cicer arietinum (chick pea)	Fetuin
20	Cytisus scoparius lectin	CSA	Scotch broom	N-Acetylgalactosamine
21	*Dolichos biflorus*	DBA	*Dolichos biflorus* (Horse Gram) seeds	αGalNAc

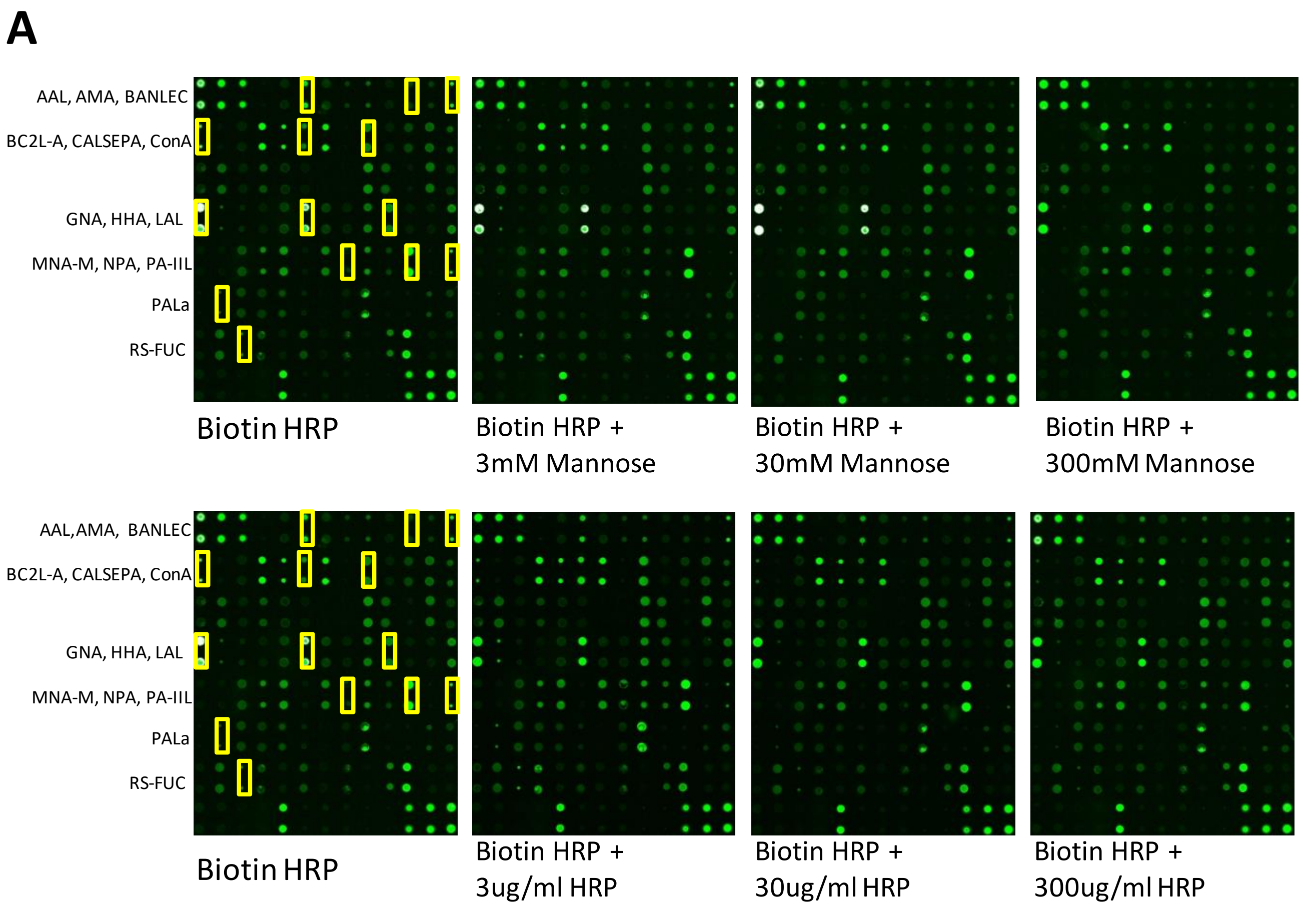
	Lectins	Abbreviation	Source	Carbonhydrate specificity
22	*Dictyostelium discoideum lectin*	Discoidin I	E. coli expressed *Dictyostelium discoideum*	αGalNAc (Tn antigen), LacNAc
23	*Dictyostelium discoideum lectin*	Discoidin II	E. coli expressed *Dictyostelium discoideum*	Gal, LacNAc, Asialoglycans, Gal/GalNAcβ1-4GlcNAcβ1-6Gal/GalNAc
24	*Datura stramonium*	DSA, DSL	*Datura stramonium* (Thorn Apple, Jimson Weed) seeds	(GlcNAc)$_{2-4}$
25	*Erythrina cristagalli*	ECA, ECL	*Erythrina cristagalli* (Coral Tree) seeds	Galβ4GlcNAc
26	*Eunonymus europaeus*	EEL	*Eunonymus europaeus* (Spindle Tree) seeds	Galα3Gal
27	*E. coli* lectin	F17AG	E. coli expressed *E. coli*	GlcNAc
28	*Pure Helix aspersa* lectin	HAA	Garden Snail	N-Acetylgalactosamine
29	*Homarus americanus* lectin	HMA	Homarus americanus (California lobster)	N-Acetylneuraminic acid, N-Acetylgalactosamine
30	Human galectin1 lectin (stable form)	Gal1	E. coli expressed human galectin1 (stable form)	branched LacNAc, Gal
31	Human galectin1-S lectin	Gal1-S	E. coli expressed human galectin1-S	branched LacNAc
32	Human galectin2 lectin	Gal2	E. coli expressed human galectin2	GalNAcα1-3Gal (Blood Group A), branched LacNAc
33	Human galectin3 lectin (full-length)	Gal3	E. coli expressed Human galectin3(full-length)	poly LacNAc
34	Human galectin 3C-S lectin	Gal3C-S	E. coli expressed Human galectin 3C-S	poly LacNAc
35	Human galectin7-S lectin	Gal7-S	E. coli expressed Human galectin7-S	Galβ1-3GlcNAc
36	Human galectin9 lectin (Stable Form)	Gal9	E. coli expressed human galectin9	poly LacNAc, GalNAcα1-3Gal (Blood Group A)
37	*Glechoma Hederacea* lectin	GHA	Glechoma hederacea (ground ivy)	Gal, methyl a-D-galactopyranoside, GalNAc
38	*Galanthus nivalis*	GNA, GNL	*Galanthus nivalis* (Snowdrop) bulbs	αMan
39	*Griffithia sp. Lectin*	GRFT	E. coli expressed *Griffithia sp.*	High-mannose
40	*Griffonia (Banderaea) simplicifolia I*	GS-I, GSL-II, BSL-I	*Griffonia (Banderaea) simplicifolia* seeds	αGal, α3GalNAc
41	*Griffonia (Brandeiraea)*	BSL-II	*Griffonia (Banderaea) simplicifolia* seeds	α or βGlcNAc
42	*Hippeastrum hybrid*	HHA, HHL, AL	*Hippeastrum* hybrid (Amaryllis) bulbs	αMan
43	*Iris hybrid lectin*	IRA		N-Acetyl-D-Galactosamine
44	Jacalin	Jacalin, AIL	*Artocarpus integrifolia* (Jackfruit) seeds	Galβ3GalNAc
45	Laburnum anagyroides lectin	LAL		a-Me-L-Fucose among monosacchrides
46	*Phaseolus lunatus*	LBA	*Phaseolus lunatus* (Lima Bean) seeds	GalNAcα(1,3)[αFuc(1,2]Gal
47	*Lens Culinaris*	LcH, LCA	*Lens culinaris* (lenti I) seeds	αMan, αGlc
48	*Lycopersicon esculentum*	LEA, LEL, TL	*Lycopersicon esculentum* (tomato) fruit	(GlcNAc)$_{2-4}$
49	Lentil lectin	Lentil	*Lens culinaris* seeds	D-Mannose, D-glucose
50	*Limulus polyphemus*	LPA	*Iris hybrid (Dutch Iris)*	Sialic Acid (N-Acetylneuraminic acid)
51	*Lotus tetragonolobus*	Lotus, LTL	*Lotus tetragonolobus, Tetragonolobus purprea(* Winged Pea, Asparagus Pea) seeds	αFuc
52	*Laetiporus sulphureus lectin*	LSL-N	E. coli expressed *Laetiporus sulphureus*	LacNAc, poly LacNAc
53	*Maackia amurensis I*	MAA, MAL, MAL-I	*Maackia amurensis* seeds	Galβ4GlcNAc
54	Human malectin lectin	Malectin	E. coli expressed human malectin	Glc$_2$-*N* -biose
55	Pure Morniga G lectin	MNA-G	Black mulberry	Galactose
56	Morniga M Lectin	MNA-M	Black mulberry	Mannose
57	*Marasmius oreades lectin*	MOA	E. coli expressed *Marasmius oreades*	3Galβ1-4GlcNAc, Galα1-3Gal
58	*Maclura pomifera*	MPL, MPA	*Maclura pomifera* (Osage Orange) seeds	Galβ3GalNAc
59	*Narcissus pseudonarcissus*	NPA, NPL, DL	*Narcissus pseudonarcissus* (Daffodil) bulbs	αMan
60	*Oryza sative lectin*	Orysata	E. coli expressed *Oryza sative*	High-mannose
61	*Pseudomonas aeruginosa lectin*	PA-IIL	E. coli expressed *Pseudomonas aeruginosa*	Fucose, Fucose containing oligosaccharides,
62	*Pseudomonas aeruginosa lectin*	PA-IL	E. coli expressed *Pseudomonas aeruginosa*	Galα1-3(4)Gal
63	*Phlebodium aureum lectin*	PALa	E. coli expressed *Phlebodium aureum*	High-mannose
64	*Phaseolus vulgaris Erythroagglutinin*	PHA-E	*Phaseolus vulgaris Erythroagglutinin* (Red Kidney Bean) seeds)	Galβ4GlcNAcβ2Manα6(GlcNAcb4)(GlcNAcβ4Manα3)Manβ4
65	*Leucoagglutinin*	PHA-L	*Phaseolus vulgaris Erythroagglutinin* (Red Kidney Bean) seeds)	Galβ4GlcNAcβ6(Gl cNAcβ2Manα3)Manα3
66	Phaseolus vulgaris agglutinin	PHA-P	*Phaseolus vulgaris Erythroagglutinin* (Red Kidney Bean) seeds)	Galβ4GlcNAcβ2Manα6(GlcNAcb4)(GlcNAcβ4Manα3)Manβ4, Galβ4GlcNAcβ6(Gl cNAcβ2Manα3)Manα3
67	Peanut	PNA	Arachis hypogaea *Peanut*	Galβ3GalNAc
68	*Pleurocybella porrigens lectin*	PPL	E. coli expressed Pleurocybella porrigens	α/βGalNAc
69	*Pisum sativum*	PSA, PEA	*Pisum sativum* (Pea) seeds	αMan, αGlc
70	*Polyporus squamosus lectin*	PSL1a	E. coli expressed *Polyporus squamosus*	α2-6 Sialic Acid
71	*Psophocarpus*	PTL, PTL-I, WBA-I	*Psophocarpus tretragonoiobus* (Winged Bean) seeds	GalNAc, Gal
72	*psophocarpus tetragonolobus lectin ii*	PTL-II, WBA-II	*Psophocarpus tretragonoiobus* (Winged Bean) seeds	anomericconfiguration), blood group H structures and the T-antigen
73	*Phytolacca americana lectin*	PWM, PWA	*Phytolacca americana* (Pokeweed)	GlcNAc(β1,4) GlcNAc oligomers, [Gal-(β1,4)
74	*Ricinus communis agglutinin ii*	RCA 60	*Ricinus communis* (Castor bean)	Galactose, N-acetyl-D-galactosamine, lactose
75	*Ricinus communis agglutinin I*	RCA 120	*Ricinus communis* (Castor bean)	galactose, lactose

	Lectins	Abbreviation	Source	Carbonhydrate specificity
76	*Robinia pseudoacacia lectin*	RPA	*black locust*	N-Acetylgalactosamine, thyroglobulin
77	*Ralstonia solanacearum lectin*	RS-Fuc	E. coli expressed *Ralstonia solanacearum*	Fucose
78	Sambucus Sieboldiana Lectin	SAMB	Japanese elderberry	NeuAcα2-6Gal/GalNAc
79	Soybean	SBA	*Glycine max* (Soybean) seeds	α > βGalNAc
80	Salivia horminum lectin	SHA	Salivia horminum	N-Acetylgalactosamine
81	*Sophora japonica*	SJA	*Sophora japonica* (Japanese Pagoda Tree)	βGalNAc
82	*Sambucus nigra I*	SNA-I	*Sambucus nigra* (Elderberry) bark	NANAα(2,6)GalNAc > GalNAc = Lac >
83	*Sambucus nigra II*	SNA-II	*Sambucus nigra* (Elderberry) bark	GalNAc > Gal
84	*Salvia sclarea lectin*	SSA	*Salvia*	N-Acetylgalactosamine
85	*Solanum tuberosum*	STL, PL	*Solanum tuberosum,* (potato) tubers	$(GlcNAc)_{2-4}$
86	*Tulipa lectin*	TL	Tulipa sp.	N-Acetylgalactosamine
87	*Urtica dioica*	UDA	*Urtica dioica* (Stinging Nettle) seeds	GlcNAc
88	*Ulex europaeus I*	UEA-I	*Ulex europaeus* (Furze Gorse) seeds	αFuc
89	*Ulex europaeus II*	UEA-II	*Ulex europaeus* (Furze Gorse) seeds	Poly β(1,4)Gl cNAc
90	*Vicia faba*	VFA	*Vicia faba* (Fava Bean) seeds	αMan
91	*Vicia villosa*	VVA, VVL	*Vicia villosa* (Hairy Vetch) seeds	GalNAc
92	*Vicia villosa*	VVA-M	*Vicia villosa* (Hairy Vetch) seeds	Mannose
93	*Vigna radiata lectin*	VRA	mung bean	a-Galactose
94	*Wisteria floribunda*	WFA	*Wisteria floribunda* (Japanese Wisteria) seeds	GalNAc
95	Wheat Germ	WGA	*Triticum volganis* (Wheat Germ)	GlCNAc

Sugar Abbreviations			
Fuc: L-Fucose	Gal: D-Galactose	GalNAc: *N*-Acetylglactosamine	Glc: D-Glucose
GlcNAc: *N*-Acetylglucosamine	Lac: Lactose	Man: Mannose	

VIII. Application 1 – Detection of Glycans on a Purified Protein

In this application, the RayBio Lectin Array 95 was used to detect specific glycosylations of purified Horseradish Peroxidase (HRP). Lectins BANLEC, BC2L-A, CALSEPA, GNA, HHA, NPA, PA-IIL, and PALa showed strong signals after incubation with 3.3 ug/mL Biotin-HRP followed by detection with streptavidin-fluorescence-dye (Figures A, B and C). The fluorescence signals from BANLEC, BC2L-A, CALSEPA, GNA, HHA, NPA, PA-IIL, and PALa were blocked in a concentration-dependent manner by HRP itself (Figures A and C), indicating that the signals were generated by lectin-HRP binding. These eight lectins are known to exhibit specific binding to mannose, which indicates that HRP contains mannose. After adding increasing amounts of mannose, the signals from BANLEC, BC2L-A, CALSEPA, GNA, HHA, NPA, PA-IIL, and PALa were reduced (Figures A and B). The reduction in signals from increasing concentrations of mannose confirms that HRP protein contains mannose in its glycocalyx. Additionally, the two lectins AAL and RS-FUC (fucose binding specificity) also showed strong interaction with HRP, which indicates the fucosylation of HRP. Overall, the results of the Lectin Array 95 were consistent with published literature regarding HRP glycosylation.



Biotin HRP

Biotin HRP +
3mM Mannose

Biotin HRP +
30mM Mannose

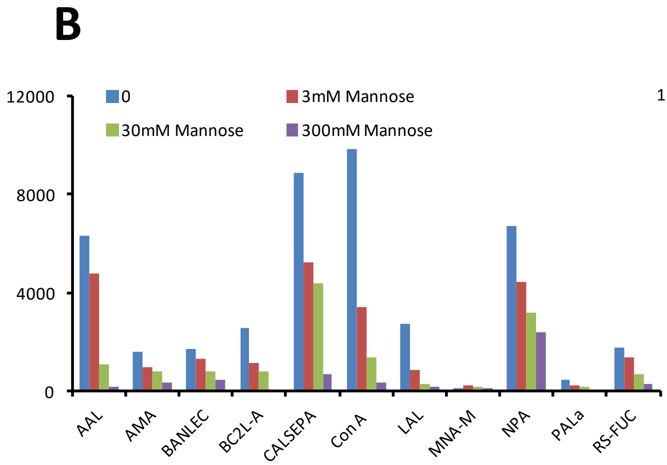
Biotin HRP +
300mM Mannose

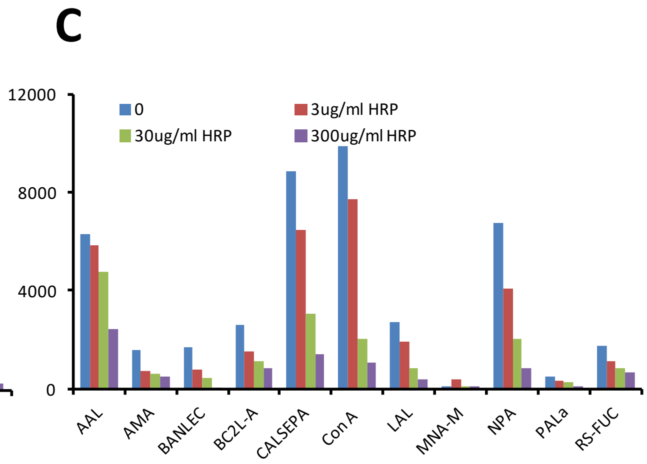
Biotin HRP

Biotin HRP +
3ug/ml HRP

Biotin HRP +
30ug/ml HRP

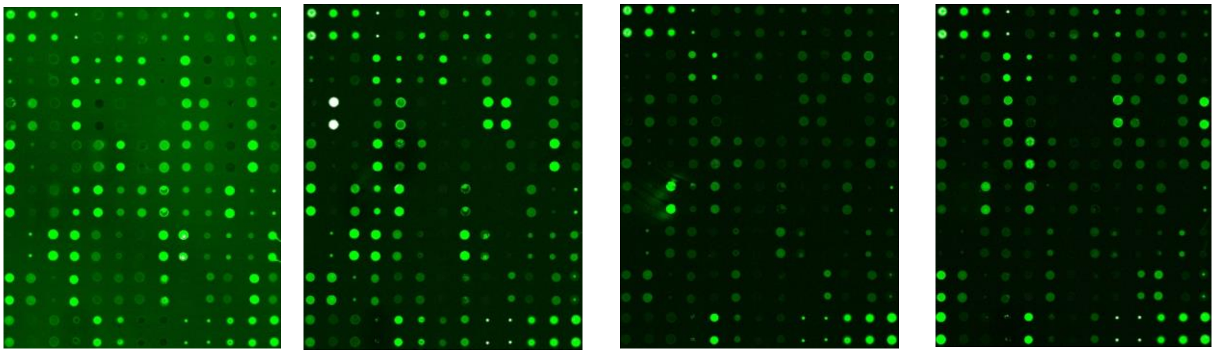
Biotin HRP +
300ug/ml HRP



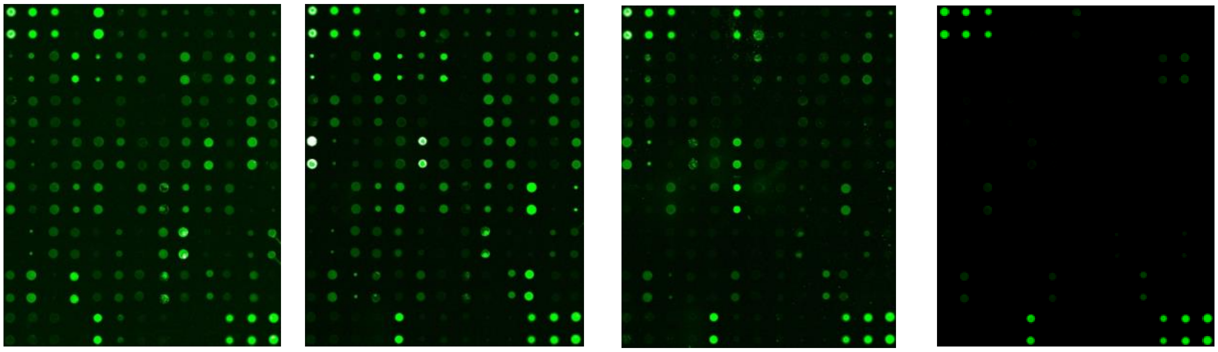


IX. Application 2 – Profiling of a Serum Sample

Using the lectin 95 array, we can discover the different glycoprotein profiles of serum samples, cell lysates, or purified glycoproteins. The images below show the profiles of the glycans from different types of samples including human serum, recombinant glycoproteins human HE4 and AFP, mouse TFF2, purified human IgG, and bacterial DE3 cell lysates detected by Biotin labeling and fluorescent dye-streptavidin.



Human Serum HE4 AFP mTFF2



Anti-Human IgG HRP DE3 Blank

X. Other Applications:

Quantitative analysis of lectin-glycoprotein interactions, Example: a concentration series of glycoproteins detected with the lectin array could reveal concentration dependent effects of lectin-glycan binding.

Determine the profile of bacterial cell-surface glycans, Example: cell lysate from bacteria can be biotinylated and hybridized to the lectin 95 array. Analysis of the binding pattern and correlation with the known carbohydrate-binding specificities of the lectins can determine the glycans present on the cell membrane.

XI. 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 completed 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 dying out during experiment

Note:

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