

RayBiotech Lectin Array 95

--Detect glycan profiles using 95 lectins

User Manual
May 10, 2018

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, Oryzata, 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

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I. Overview

A. Introduction

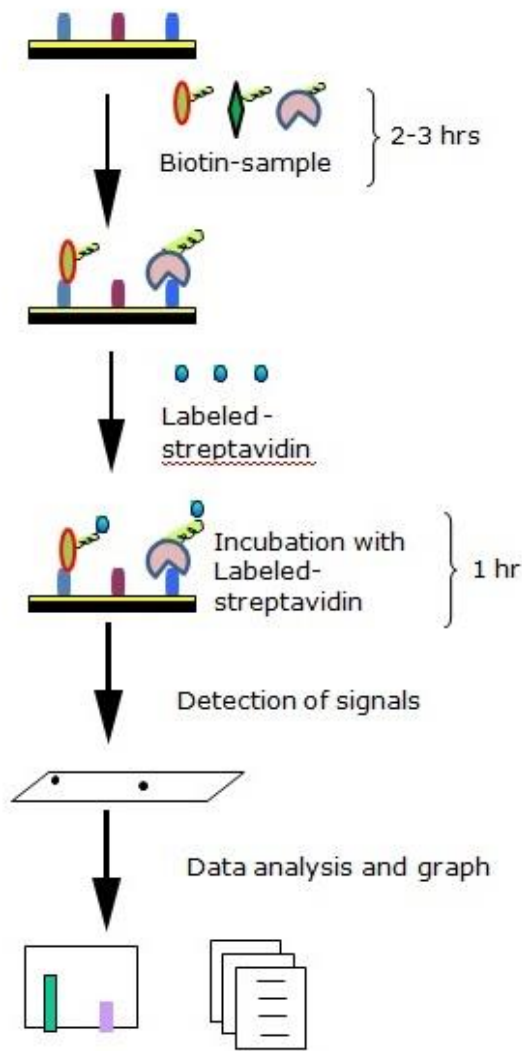
Glycocalyx, literally meaning 'sugar coat', is an extracellular polymeric coating surrounding many prokaryotic and eukaryotic cells consisting of glycoproteins, glycolipids, proteoglycans and glycosaminoglycans. The constituents of the glycocalyx play an important role in the process of cell signaling, 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.

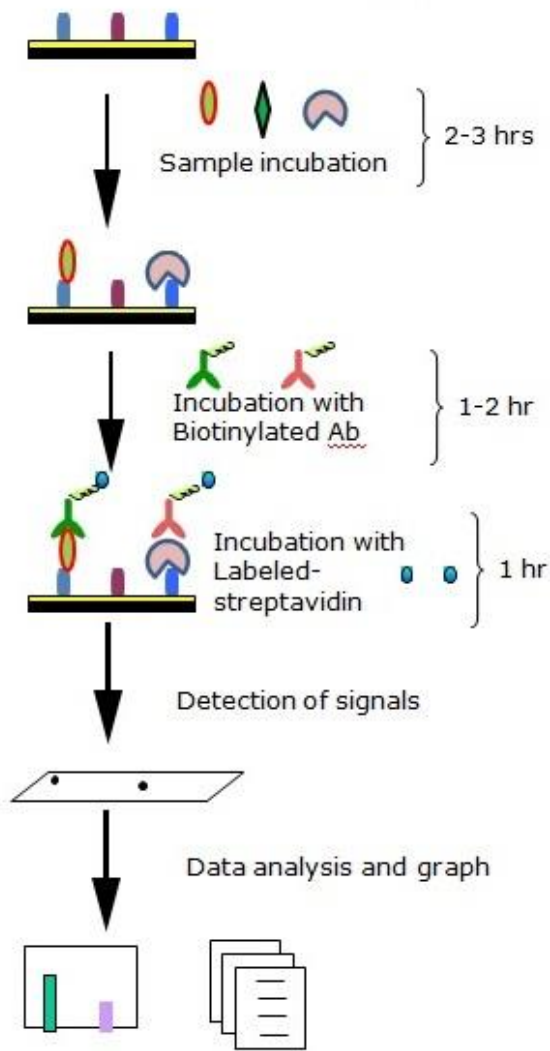
Lectins are glycan-binding proteins which have been purified from trees, beans and some fruits. They are highly specific for a given glycan based on their sequence and the different sugar unit structures the glycan contains. Based on the previously launched Lectin Array 40 and Lectin Array 70, RayBiotech 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

Label-based Approach



Sandwich-based Approach



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 2, 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

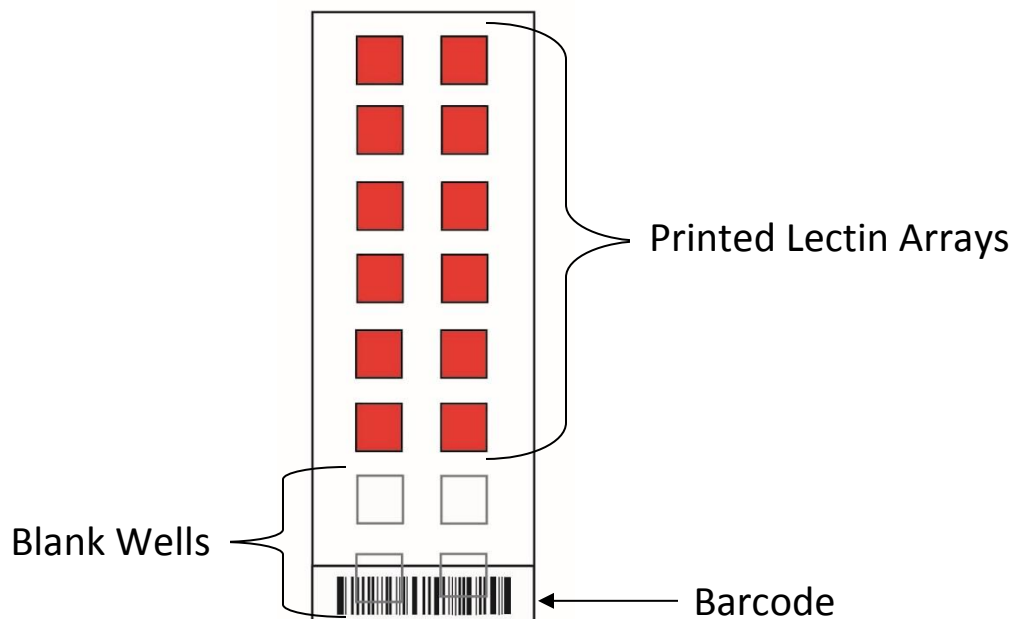
Item	Description	1-Slide kit	2-Slide kit	4-Slide kit
1	Dialysis Vials	24	48	96
2	Labeling Reagent	4	8	16
3	Labeling Buffer	1	2	4
4	Stop Solution	1	2	4
5	Lectin Array Glass Slide Assembly	1	2	4
6	Sample Diluent	1	1	2
7	20X Wash Buffer I	1	1	2
8	20X Wash Buffer II	1	1	2
9	Cy3 equivalent dye-conjugated Streptavidin	1	2	4
10	Slide Washer/Dryer	1	1	2
11	Adhesive device sealer	2	4	8
12	Floating Dialysis Rack	2	4	8
13	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, KH₂PO₄ and Na₂HPO₄ (For label-based method only)
- Plastic or glass containers, beaker, stir plate and stir bar
- Pipettors, pipette tips, ddH₂O 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 glyocalyx elements (glycolipids, glycoproteins, etc.) captured on the array. The user will need to supply the labeled reporter antibodies specific for the glyocalyx 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 μ l of original or diluted serum, plasma, cell culture media, or other body fluid, or 50-100 μ l 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°C. 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 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 Vial (Item 1). 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 2). 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 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 3).
- c. For labeling cell or tissue lysates: transfer 30 μ g (for example, 15 μ l of 2 mg/ml) cell or tissue lysates into a tube and add Labeling Buffer (Item 3) 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.

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 4) 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 100 μ l Sample Diluent (Item 6) 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°C 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 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 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 μ l 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

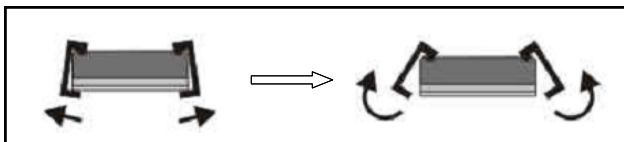
Note: For the Sandwich-based protocol, follow below steps after secondary antibody incubation if biotinylated secondary antibody is used. If fluorescence conjugated secondary antibody is used, skip steps 13-15 and continue from step 16 after incubation of secondary antibody. Appropriate dilution of secondary 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 μ l 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 μ l 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 N₂ 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 I	DSA	ECA	EEL	F17AG	GAL1	GAL1-S	GAL2	GAL3	GAL3C-S	GAL7-S	GAL9	GHA
6	DISCOIDIN I	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	<i>Anguilla anguilla</i>	AAA	<i>Anguilla anguilla</i> (Fresh Water Eel)	α Fuc
2	<i>Aleuria aurantia</i>	AAL	<i>Aleuria aurantia</i> mushrooms	Fuc α 6GlcNAc
3	<i>Agaricus bisporus</i> lectin	ABA	<i>Agaricus bisporus</i>	Galactose (β 1,3) N-Acetylgalactosamine
4	<i>Agaricus bisporus</i> lectin	ABL	<i>Agaricus bisporus</i> (White button mushroom)	galactose- β -1,3-N-acetylgalactosamine, galactose- β -1,3-N-acetylglucosamine
5	<i>Agrocybe cylindracea</i> lectin	ACG	E. coli expressed <i>Agrocybe cylindracea</i> galectin lectin	α 2-3 Sialic Acid
6	<i>Amaranthus caudatus</i>	ACL, ACA	<i>Amaranthus caudatus</i> seeds	Gal β 3GalNAc
7	<i>Arum maculatum</i> lectin	AMA	<i>Arum maculatum</i> (Lords and Ladies)	Mannose
8	<i>Allium sativum</i>	ASA	<i>Allium sativum</i> agglutinin (Garlic)	α Man
9	<i>Musa acuminata</i> lectin	BanLec	E. coli expressed <i>Musa acuminata</i>	containing α 1,3-glycoside bond
10	<i>Burkholderia cenocepacia</i> lectin	BC2L-A	E. coli expressed <i>Burkholderia cenocepacia</i>	High-mannose
11	<i>Burkholderia cenocepacia</i> lectin	BC2LCN (AiLe cS1)	E. coli expressed <i>Burkholderia cenocepacia</i>	Fuc α 1-2Gal β 1-3GalNAc (H type 3), Fuc α 1-2Gal β 1-3GlcNAc (H type 1)
12	<i>Bauhinia purpurea</i>	BPA, BLP	<i>Bauhinia purpurea alba</i> (Camel's Foot Tree)	Gal β 3GalNAc
13	<i>Colchicum autumnale</i>	CA	<i>Colchicum autumnale</i>	Lactose > N-Acetylgalactosamine > Galactose
14	<i>Caragana arborescens</i>	CAA	<i>Caragana arborescens</i> (pea tree)	N-Acetylgalactosamine
15	<i>Calystegia sepium</i> lectin	Calsepa	E. coli expressed <i>Calystegia sepium</i>	High-mannose
16	<i>Coprinopsis cinerea</i> lectin	CGL2	E. coli expressed <i>Coprinopsis cinerea</i>	β Gal, GalNAc α 1-3Gal (Blood Group A), Gal α 1-3Gal (Blood Group B)
17	<i>Clitocybe nebularis</i> lectin	CNL	E. coli expressed <i>Clitocybe nebularis</i>	2]Gal β 1-4GlcNAc (Blood Group A)
18	Conavalin A	Con A	<i>Conavalia ensformis</i> (Jack Beans) seeds	α Man, α Glc
19	<i>Cicer arietinum</i> lectin	CPA	<i>Cicer arietinum</i> (chick pea)	Fetuin
20	<i>Cytisus scoparius</i> lectin	CSA	Scotch broom	N-Acetylgalactosamine
21	<i>Dolichos biflorus</i>	DBA	<i>Dolichos biflorus</i> (Horse Gram) seeds	α GalNAc

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

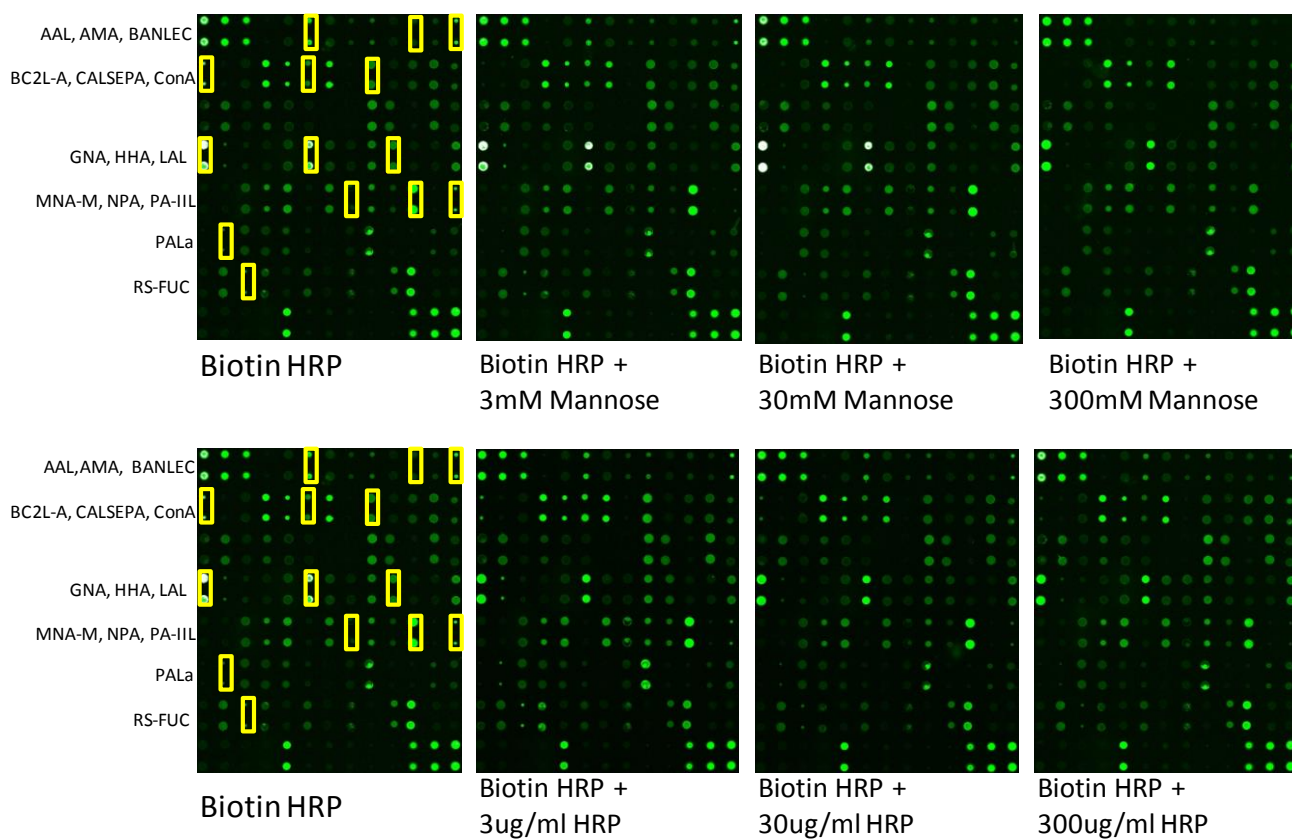
	Lectins	Abbreviation	Source	Carbohydrate specificity
76	<i>Robinia pseudoacacia</i> lectin	RPA	<i>black locust</i>	N-Acetylglactosamine, thyroglobulin
77	<i>Ralstonia solanacearum</i> lectin	RS-Fuc	<i>E. coli</i> expressed <i>Ralstonia solanacearum</i>	Fucose
78	<i>Sambucus Sieboldiana</i> Lectin	SAMB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

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

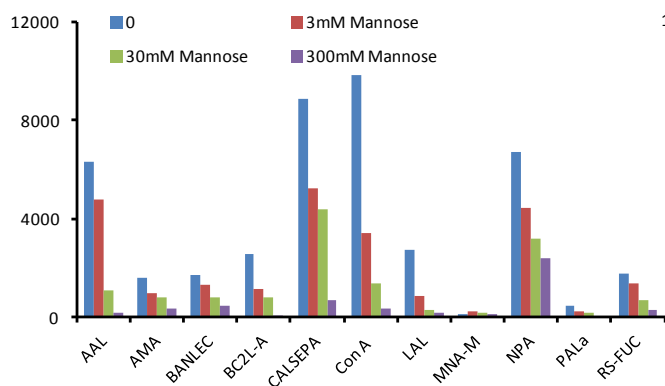
VIII. Application 1 – Detection of Glycans on a Purified Protein

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.

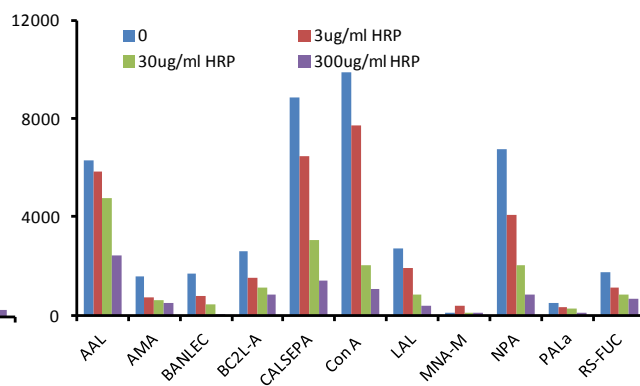
A



B



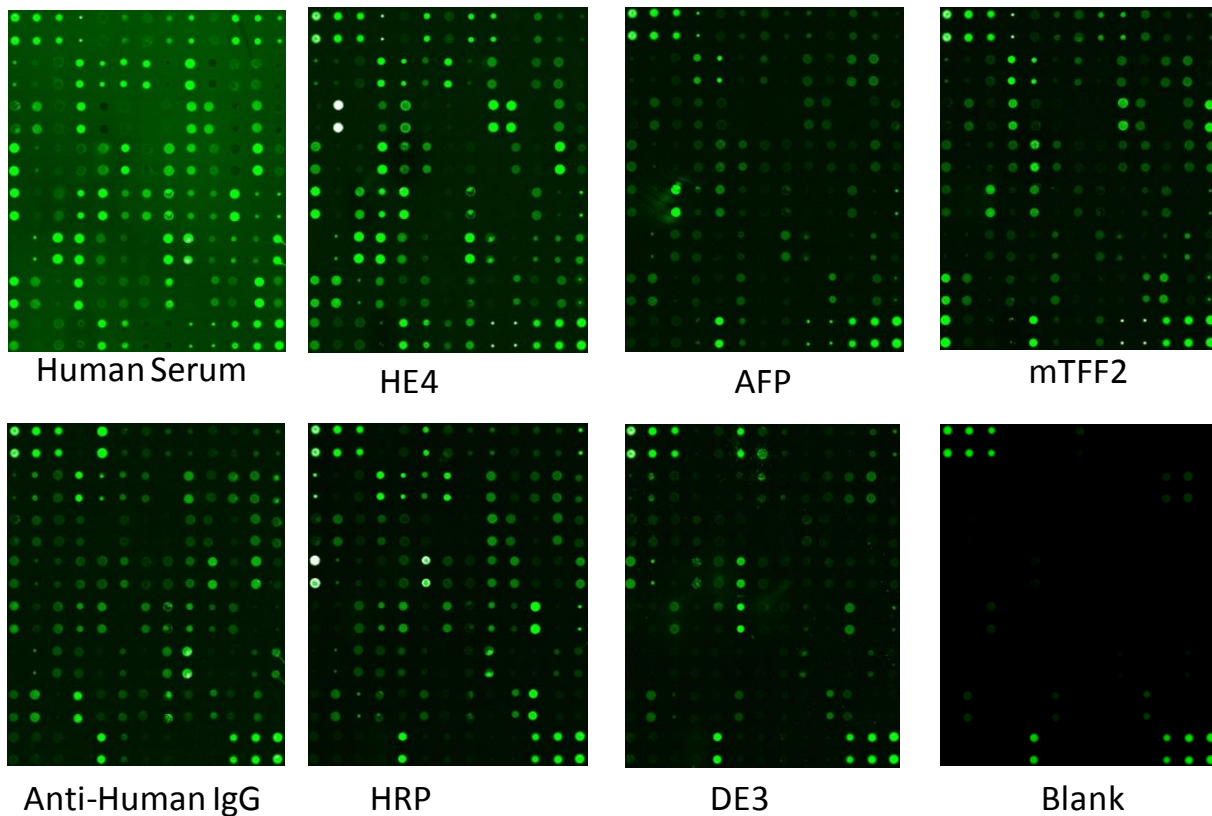
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IX. Application 2 – Profiling of a Serum Sample

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.



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 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

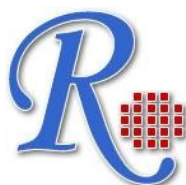
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