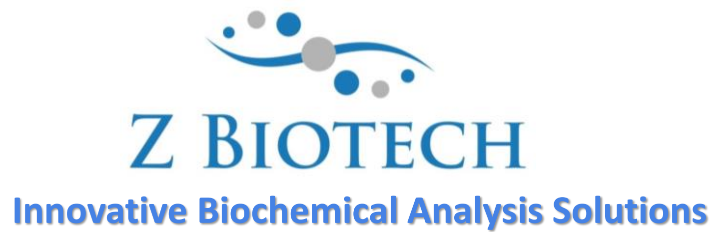
Lectin Microarray User Manual



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Introduction

Lectins are a class of carbohydrate-binding proteins purified from plants or animals that can bind to different carbohydrate structural epitopes in a highly specific manner. We selected 26 lectins to include in our first-generation lectin microarray. These 26 lectins represent most carbohydrate-binding epitopes and have been characterized on our glycan arrays in order to offer the most established information about lectin binding. There are 8 or 16 identical subarrays on a single array chip so that 8 or 16 samples can be analyzed simultaneously. Our lectin microarray provides scientists with a powerful and sensitive tool for analyzing glycosylation profiles of therapeutic proteins, biomarkers, or other proteins of interest.

The applications for the lectin microarray include:

- Analysis of glycosylation profiles of proteins, antibodies, cells, and cell lysates.
- Carbohydrate biomarker discovery and analysis.
- Comparison of glycosylation pattern differences or alterations.
- Identification of aberrantly glycosylated cells, proteins, or antibodies.

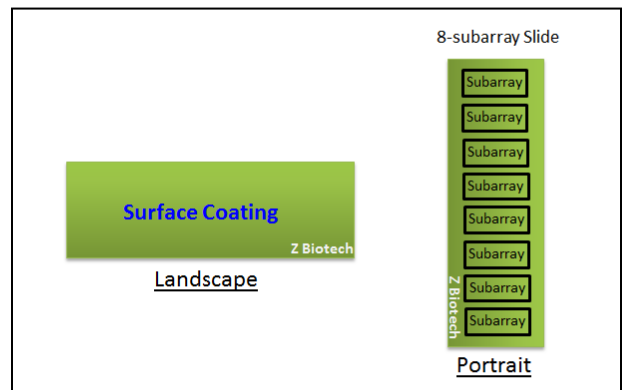
Handling and Storage

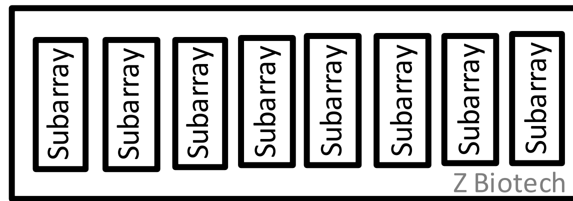
Store the bag of slides and any buffers in a 4°C refrigerator if they are to be assayed within 3 weeks upon receipt. For long term storage keep the bag of slides at -20°C. Avoid freezing and thawing multiple times. Purchased slides and buffers should be used within 6 months.

Allow the bag of slides to equilibrate to room temperature at least 20 minutes before opening. After opening, re-seal any unused slides in the moisture barrier bag with a desiccant inside and refreeze.

Array Map/Schematic

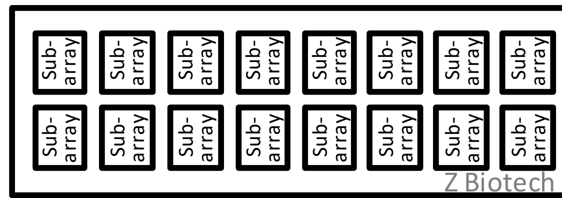
Lectin Microarray slides have either 8 or 16 subarrays. Arrays are printed on the side with the "Z Biotech" label facing upward. The "Z Biotech" label is located on the bottom right corner from a landscape point of view, or the bottom left corner in a portrait point of view (see image on right). The orientation of the printed array is consistent with the portrait slide orientation. Dimensions and array map are shown:





8-subarray Array Layout

L1	L1	L1	L1	L1	L1	L2	L2	L2	L2	L2	L2	L3	L3	L3	L3	L3	L3	L4	L4	L4	L4	L4	L4
L5	L5	L5	L5	L5	L5	L6	L6	L6	L6	L6	L6	L7	L7	L7	L7	L7	L7	L8	L8	L8	L8	L8	L8
L9	L9	L9	L9	L9	L9	L10	L10	L10	L10	L10	L10	L11	L11	L11	L11	L11	L11	L12	L12	L12	L12	L12	L12
L13	L13	L13	L13	L13	L13	L14	L14	L14	L14	L14	L14	L15	L15	L15	L15	L15	L15	L16	L16	L16	L16	L16	L16
L17	L17	L17	L17	L17	L17	L18	L18	L18	L18	L18	L18	L19	L19	L19	L19	L19	L19	L20	L20	L20	L20	L20	L20
L21	L21	L21	L21	L21	L21	L22	L22	L22	L22	L22	L22	L23	L23	L23	L23	L23	L23	L24	L24	L24	L24	L24	L24
L25	L25	L25	L25	L25	L25	L26	L26	L26	L26	L26	L26	NC	NC	NC	NC	NC	NC	PC	PC	PC	PC	PC	PC
																		M	M	M	M	M	M



16-subarray Array Layout

L1	L1	L1	L2	L2	L2	L3	L3	L3	L4	L4	L4
L5	L5	L5	L6	L6	L6	L7	L7	L7	L8	L8	L8
L9	L9	L9	L10	L10	L10	L11	L11	L11	L12	L12	L12
L13	L13	L13	L14	L14	L14	L15	L15	L15	L16	L16	L16
L17	L17	L17	L18	L18	L18	L19	L19	L19	L20	L20	L20
L21	L21	L21	L22	L22	L22	L23	L23	L23	L24	L24	L24
L25	L25	L25	L26	L26	L26	NC	NC	NC	PC	PC	PC
									Marker	Marker	Marker

<u>Controls</u>

NC: negative control (Print Buffer)

PC: positive control (a biotinylated probe)

M: Marker

Lectin Identification List

ID	Lectin Short Name	Lectin Full Name	Specificity Group
L1	ConA	*Canavalia ensiformis* Concanavalin A	Core Man3 (Manα1-6(Manα1-3)Manβ1-4); ≥3-sugar α-glucan chains; weak partial-epitope binding.
L2	GNA	*Galanthus nivalis* agglutinin	Exposed α1,3-Man
L3	PHA-L	*Phaseolus vulgaris* Leukoagglutinating Phytohemagglutinin	Tri/Tetra-antennary N-Glycans
L4	PHA-E	*Phaseolus vulgaris* Erythroagglutinating Phytohemagglutinin	Complex N-Glycans; no high-mannose binding
L5	DSA	*Datura stromonium* Agglutinin	Type 2 LacNAc; $(GlcNAc\beta1\text{-}4)_2$ and some extended epitopes.
L6	LCA	*Lens culinaris* Agglutinin	N-Glycan core: GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc; core fucose (Fucα1-6GlcNAc) enhances binding; no binding to bisecting or tri-antennary glycans; high-mannose with GlcNAc at reducing end also binds.
L7	MAL-I	*Maackia amurensis* Leukoagglutinin	Terminal Neu5Ac(or Neu5Gc)α2-3Galβ1-4GlcNAc(or Glc); weak asialo partial-epitope binding; no SLe^{X} or Sd^{a} antigen binding; terminal α2,8-Neu5Ac does not block binding.
L8	MAL-II	*Maackia amurensis* Hemagglutinin	
L9	SNA	*Sambucus nigra* Agglutinin	Terminal Neu5Ac(or Neu5Gc)α2-6Galβ1-4; no asialo partial-epitope binding
L10	AAL	*Aleuria aurantia* Leukoagglutinin	Fucose-containing glycans; some blood-group antigens may not bind.
L11	UEA-I	*Ulex europeus* Agglutinin-I	Fucα1-2Galβ1-4GlcNAc; Lewis Y; α1,4-Fuc
L12	LTA	*Lotus tetragonolobus* Agglutinin	Terminal Fucα1-3GlcNAc; Lewis X; Lewis Y; Fucα1-2Galβ1-4
L13	RCA-I	*Ricinus communis* agglutinin-I	Terminal β1,4-Gal (β1,3 and β1,6 also accepted); no binding with terminal α2,3 sialic acid, or to T antigen or Lewis X; still binds to β1,4-Gal capped with terminal α2,6 sialic acid.
L14	ECL	*Erythrina cristagalli* Lectin	Terminal β1,4-Gal; no binding with terminal sialic acid or to Lewis X.
L15	PNA	*Arachis hypogaea* Peanut agglutinin	Galβ1-3GalNAc (T antigen); GD1b; GM1; Gb5; iGb5.
L16	*Jacalin*	*Jacalin*	Terminal αGalNAc
L17	GSI-B4	*Griffonia (Bandeiraea) simplicifolia* Agglutinin I B4	Terminal α1,3-Gal (or α1,4-Gal)
L18	WFA	*Wisteria floribunda* Lectin	β-GalNAc > α-GalNAc > terminal β-Gal (no binding to Lewis X); Sd^{a} antigen; no T antigen binding.
L19	SBA	*Glycine max* Soybean Agglutinin	Terminal αGalNAc > βGalNAc > αGal
L20	HPA	*Helix pomatia Lectin*	Terminal αGalNAc > βGalNAc > αGal; weak binding to T antigen; no binding to Sd^{a} antigen
L21	VVL	*Vicia villosa* Lectin	Terminal α-GalNAc or β-GalNAc
L22	DBA	*Dolichus biflorus* Agglutinin	Exposed and terminal βGalNAc > αGal; Sd^{a} antigen; no T antigen binding.
L23	GSII	*Griffonia (Bandeiraea) simplicifolia* Agglutinin II	Terminal GlcNAc (β or α linkage); terminal βGalNAc; no binding with 6S sulfation
L24	WGA	*Triticum vulgaris* Wheat germ Agglutinin	GlcNAc; terminal and exposed α2,3-Neu5Ac; terminal and exposed α-GalNAc; no binding to Lewis X; no binding with terminal α2,6 sialic acid.
L25	LEL	*Lycopersicon esculentum* (tomato) lectin	Neu5Ac(or Neu5Gc or Kdn)α2-3Galβ1-4GlcNAc (type 2 sialyl poly-LacNAc); weak partial-epitope binding.
L26	STL	Solanum tuberosum Potato Lectin	Galβ1-4GlcNAc (type 2 poly-LacNAc); weak partial-epitope binding.

Materials Required

- Arrayed glass slide
- 16 or 8 subarray cassettes
- Blocking Buffer (NGBB Item # 10106)
- Lectin Array Assay Buffer (LAAB Item #10111)
- Wash Buffer: 20 mM Tris-HCl, 150 mM NaCl, 0.05% Tween 20, pH 7.6
- Glycoprotein samples of interest

- Detection antibodies specific to your glycoprotein samples (or a labeled fluorophore if your samples are tagged).
- Laser fluorescence scanner (able to scan at the wavelength of your fluorophore)
- Coplin jar
- Adhesive slide cover film

Preparation of assay samples:

Prepare glycoprotein samples or detection antibodies in a centrifuge tube by diluting with the LAAB buffer. For the fluorescently labelled streptavidin we recommend a concentration of 1 µg/mL. For detection antibodies, we suggest a concentration around 10-1 µg/ml. A range of 100 µg/ml to 0.1 µg/ml concentration for glycoprotein samples works, although some experimentation may be required to establish the concentration that will provide the highest binding signals with the lowest background fluorescence. This can be accomplished by applying a different dilution of samples to different wells of the array. In addition to testing a dilution range for your glycoprotein of interest, we recommend setting up control assays for any additional detection or secondary antibodies (including streptavidin) to ensure that any binding observed is specific to your glycoprotein of interest. A fluorescent signal due to specific binding to your glycoprotein of interest should be dose-dependent within the dynamic range of your protein dilution, and should have a positive binding signal after a signal from negative control spots and control assays has been subtracted. Calculate the volume of sample needed depending on how many slides and subarrays are to be assayed. Use 100 µL volume of sample per well for 16 subarray cassettes and 200 µL for 8 subarray cassettes to ensure full and even coverage of the printed area throughout incubation. If necessary, the assay can be done successfully with a minimal volume of 60 µL per well for 16 subarray cassettes and 100 µL for 8 subarrays. Using a minimal volume in the wells has an increased risk of the array drying out during the assay, and may also cause unequal distribution of the sample across the arrayed surface which may result in signal variation. Please ensure each sample is homogeneous and thoroughly mixed.

Assay Protocol

Part 1 – Blocking

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

1. Allow the arrayed slides to equilibrate to room temperature (20-30 minutes) before opening the moisture barrier bag.
2. Add blocking buffer to each subarray well. We recommend using 100 µL per well for 16 subarray cassettes and 200 µL for 8 subarray cassettes in order to ensure full and even coverage of the printed area throughout incubation.
3. Cover the wells with adhesive film to prevent evaporation and incubate slide on shaker at 85 rpm for 1 hour. Longer incubation time is acceptable, but not necessary.

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

Part 2 – Binding assay

1. Unless the glycoprotein sample of interest is bacteria or cells, centrifuge samples briefly to avoid adding irrelevant or aggregated particles to the array.
2. Touch the pipette tip to the corner of the well of the cassette and tip the slide so that the sample pools to that corner to remove blocking buffer. Avoid touching the array surface.
3. Briefly rinse each well with assay buffer and then remove it by pipette.

4. Immediately apply the glycoprotein sample of interest to each well. We recommend using 100 µL per well for 16 subarray cassettes and 200 µL for 8 subarray cassettes in order to ensure full and even coverage of the printed area throughout incubation. Avoid leaving air bubbles.
5. Seal the wells with adhesive film to prevent evaporation. If the sample is fluorescently labelled, cover with aluminum foil to keep it in the dark. Incubate on the shaker for 1-3 hours at 100 rpm. Longer incubation time may increase binding signal, especially for weakly binding samples.

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

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

Part 3 – Wash

1. Remove lectin-binding samples from each well by gently touching the pipette tip to the corner of the well of the cassette and tip the slide so that the sample pools to that corner. Avoid touching the array surface, but a gentle touch is okay to ensure no sample is left pooled in the corners.
2. Add wash buffer to each well. We recommend using 100 µL per well for 16 subarray cassettes and 200 µL for 8 subarray cassettes. Cover the wells with adhesive film and incubate on the shaker for 5 minutes at 85 rpm. Completely remove the wash buffer by pipette and repeat this step. Avoid allowing the slide to dry out and have your next wash or sample ready before you remove the wash buffer.

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

Part 4 – Binding of biotinylated antibody (Sandwich Assay Format)

1. Unless the secondary biotinylated antibody sample is bacteria or cells, centrifuge samples briefly to avoid adding irrelevant particles to the array.
2. After completely removing the wash buffer immediately add of the biotinylated antibody to each well. We recommend using 100 µL per well for 16 subarray cassettes and 200 µL for 8 subarray cassettes. Seal the wells with adhesive film and incubate on the shaker for 1 hour at 100 rpm. Longer incubation time is acceptable, but not necessary.
3. After incubation repeat Part 3 – Wash.

Part 5 – Fluorescent staining

1. Centrifuge fluorescent labeled streptavidin samples briefly to avoid adding irrelevant particles to the array.
2. After completely removing the wash buffer immediately add the fluorescently labelled streptavidin sample. 100 µL per well is recommended for 16 subarray cassettes and 200 µL for 8 subarray cassettes. Seal the wells with adhesive film and shield the wells from light with aluminum foil. Incubate on the shaker at 85 rpm for 1 hour. Longer incubation time is acceptable, but not necessary.

Part 6 – Final wash and dry

1. Touch the pipette tip to the corner of the well of the cassette and tip the slide so that the sample pools to that corner to remove it. Avoid touching the array surface.
2. Briefly rinse each well with wash buffer. 100 µL per well is recommended for 16 subarray cassettes and 200 µL for 8 subarray cassettes.
3. Completely remove the wash buffer by pipette. Avoid touching the array surface. Repeat steps 2 and 3.
4. Disassemble the cassette from the slide. For the provided cassette this can be done by holding the slide with one hand at the top and bottom edges and sliding out the cassette clips one by one with the other hand. If your provided cassette has metal clips, they can be removed by rotating the clip outwards from the bottom of the slide. When the clips have been removed place the slide on the table and hold a small outer edge of the slide to the table as you gently peel the cassette off.
5. Immediately immerse the slide in a coplin jar or beaker full of wash buffer. Do not touch the surface of the array or allow the array surface to touch the sides of the beaker or jar.
6. Place the jar or beaker on the 60 rpm shaker for 10 minutes.
7. Decant the wash buffer from the jar or beaker while holding the slide in place (only touch the edge of the slide) and then add sterile de-ionized water to immerse the slide.
8. Place the jar or beaker on the 60 rpm shaker for 2 minutes.
9. Decant the water from the jar or beaker.
10. Allow the slide to dry completely in a clean, dust free environment before scanning.

Analysis

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

Interpretation of Control Signals:

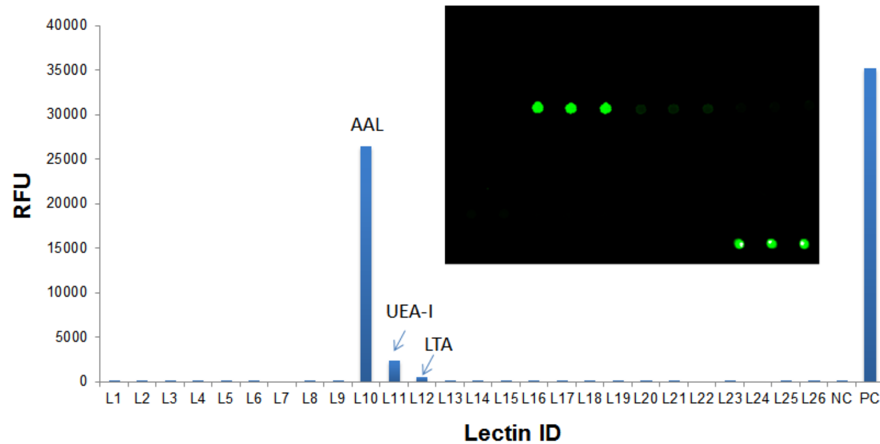
<u>Negative Control (Print Buffer):</u> The negative control should produce a signal close to the intensity of the background. Since there is no binding involved with the negative control, any other signals around the negative control's intensity are also not binding. Subtracting the negative control's signal from the other binding signals will give more accurate specific binding data.

<u>Positive Control (a biotinylated probe):</u> This positive control will bind directly to the fluorescent labelled streptavidin. If your glycoprotein sample is already fluorescently labelled, or in any case where the addition of fluorescent labelled streptavidin to the array was not preformed (Part 5 – Fluorescent staining) this positive control will not be reactive.

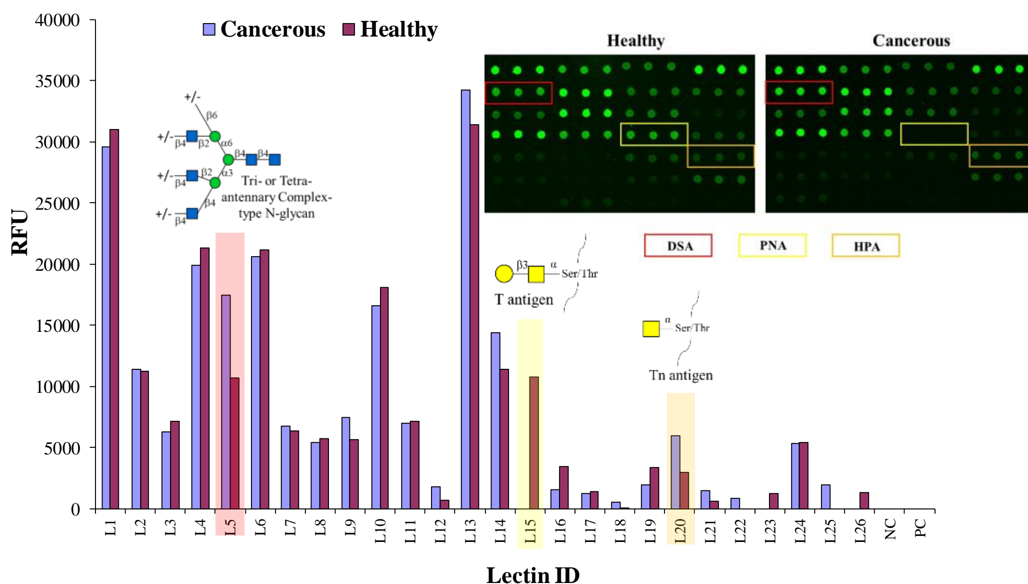
Typical Binding Assay Result from the Lectin Microarray

Example 1: Lectin Microarray on 16 subarray formats. A subarray assayed with a biotinylated α-L-fucose target (0.01 µg/ml), followed by streptavidin-Cy3 (1 µg/ml). The array was scanned with GenePix scanner at 500 PMT and 100% laser

power at 532nm wavelength. The positive control shows binding as expected. Three lectins, AAL, UEA-I and LTA, show specific binding to the target.

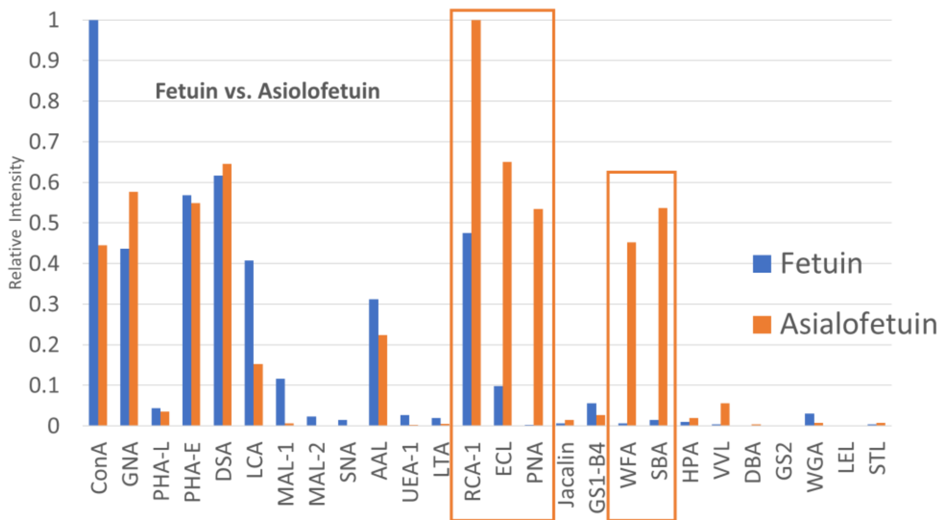


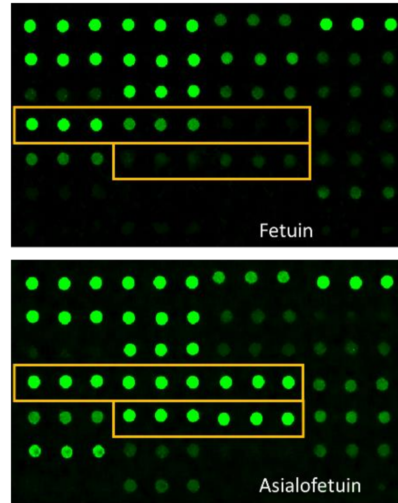
Example 2: Lectin Microarray on 8 subarray formats. Two subarrays assayed with AlexaFluor555-labeled cancerous (breast cancer) and healthy human serum (1:50 dilution). The array was scanned with GenePix scanner at 450 PMT and 100% laser power at 532nm wavelength. Data is normalized by equalizing the sum of the 26 lectin intensities on the cancerous and healthy sample subarrays. There is no binding to positive control because the samples were pre-labeled. By comparing healthy to cancerous sera on this array we can observe differences in glycosylation patterns. One such observation is that the PNA lectin (L15) binding is relatively low in the cancerous sample, indicating that its known binding epitope – T antigen – is hypo-expressed in the cancerous serum. There is also relatively increased HPA lectin (L20) binding in the cancerous sample, indicating that its known binding epitope – Tn antigen – is more prevalent. One explanation for this could be a mutation of the gene encoding Cosmc in the cancer patient. Cosmc is a chaperone required for the expression of the enzyme that synthesizes T antigen from Tn antigen, and a deficiency in this pathway would result in more Tn antigen and less T antigen. In addition, there is increased DSA lectin (L5) binding in the cancerous sample, indicating elevated tri- or tetra-antennary complex-type N-glycans. This suggests an overexpression of GlcNAcT-IV, the enzyme that mediates the biosynthesis of tri- or tetra-antennary N-glycans by adding a 4-linked GlcNAc onto the 3-mannose arm.



Example 3: Lectin Microarray on 16 subarray formats. Two subarrays assayed with AlexaFluor555-labeled fetuin glycoprotein and asialofetuin glycoprotein (50 µg/ml). The array was scanned with InnoScan scanner at 1 PMT and low

laser power at 532nm wavelength. Data is normalized by the signal of the lectin with the highest binding affinity. There is no binding to positive control (a biotinylated probe) because the samples were labeled with fluorescence. By comparing fetuin and asialofetuin on this array we can observe differences in glycosylation (sialylation) patterns. The clearest observation is that there is a relative increase in binding to strong galactose binders (i.e., RCA-1, ECL, PNA, WFA, SBA) for asialofetuin. As expected, this indicates more exposed galactose epitopes where there lacks a sialic acid terminus.





Troubleshooting

Condition	Possible Causes	Potential Solutions
High Background	• Concentration of protein samples is too high. • Concentration of fluorescent samples is too high. • Arrays are not thoroughly washed. • Slide drying out during assay. • Excessive particles in the samples due to sample aggregation, dust, etc.	• Use a lower concentration range of samples. Consider a wider range if you are unsure where the detection limit is. Use control assays to determine which sample is causing high background. • Apply longer times for washing steps and use a higher shaking rate • Make sure wash buffer and sample is completely removed before the next step. • Make sure adhesive film fully seals the wells to avoid evaporation • Centrifuge the samples prior to assay to avoid adding irrelevant particles. Make sure buffers are filtered. • If you think that the protein is aggregating during incubation, try shaking at a higher speed

Signal Variation	• Slide drying out during assay. • Binding samples are not equally distributed in the wells • Glycan-binding protein aggregation during incubation • Bubbles during incubation	• Make sure wells are sealed to prevent evaporation during incubation. • Apply a larger volume of sample to each well to ensure equal distribution • Use a higher shaking rate during incubation • Make sure samples are homogeneous, mixed thoroughly, and do not leave bubbles on the array surface
Unexpected Binding	• Cross contamination between wells or other sources. • Sample contamination	• Make sure to use sterilized pipette tips and tubes used for sample application and preparation • Ensure cassette is pressed firmly to the slide so that there are no gaps to allow leaking between wells • Be careful not to cross contaminate samples when applying to the wells, even during wash steps