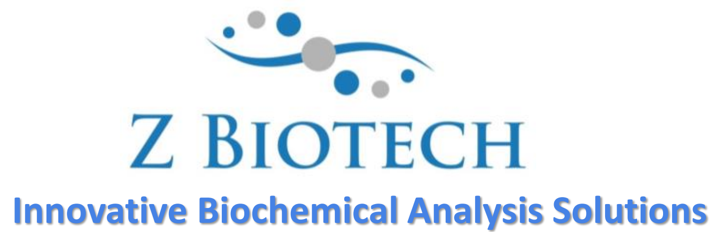
Lectin Microarray User Manual



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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 38 lectins to include in our second-generation lectin microarray. These 38 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.

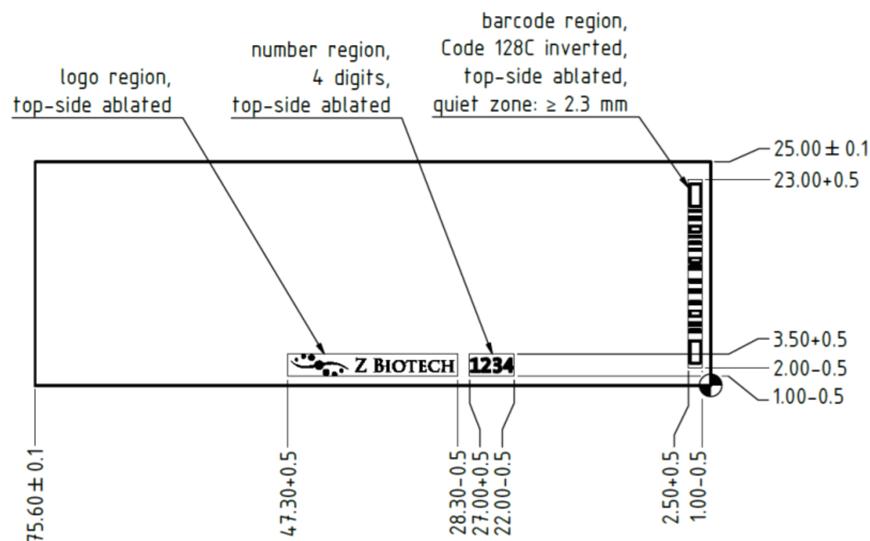
Handling and Storage

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 and 4-digit number ID facing upward. The "Z Biotech" label is located on the bottom center from a landscape view. The number ID is consistent with the barcode ID on the bottom from a portrait view. Dimensions and array maps are shown below.



8-subarray Array Layout

8-subarray Slide

Subarray Subarray Subarray Subarray Subarray Subarray Subarray Subarray

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NL1	NL1	NL1	NL1	NL1	NL1	NL2	NL2	NL2	NL2	NL2	NL2	NL3	NL3	NL3	NL3	NL3	NL3	NL4	NL4	NL4	NL4	NL4	NL4	NL5	NL5	NL5	NL5	NL5	NL5
NL6	NL6	NL6	NL6	NL6	NL6	NL7	NL7	NL7	NL7	NL7	NL7	NL8	NL8	NL8	NL8	NL8	NL8	NL9	NL9	NL9	NL9	NL9	NL9	NL10	NL10	NL10	NL10	NL10	NL10
NL11	NL11	NL11	NL11	NL11	NL11	NL12	NL12	NL12	NL12	NL12	NL12	NL13	NL13	NL13	NL13	NL13	NL13	NL14	NL14	NL14	NL14	NL14	NL14	NL15	NL15	NL15	NL15	NL15	NL15
NL16	NL16	NL16	NL16	NL16	NL16	NL17	NL17	NL17	NL17	NL17	NL17	NL18	NL18	NL18	NL18	NL18	NL18	NL19	NL19	NL19	NL19	NL19	NL19	NL20	NL20	NL20	NL20	NL20	NL20
NL21	NL21	NL21	NL21	NL21	NL21	NL22	NL22	NL22	NL22	NL22	NL22	NL23	NL23	NL23	NL23	NL23	NL23	NL24	NL24	NL24	NL24	NL24	NL24	NL25	NL25	NL25	NL25	NL25	NL25
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NL36	NL36	NL36	NL36	NL36	NL36	NL37	NL37	NL37	NL37	NL37	NL37	NL38	NL38	NL38	NL38	NL38	NL38	NC	NC	NC	NC	NC	NC	PC1	PC1	PC1	PC1	PC1	PC1
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	M	M	M	M	M	M

16-subarray Array Layout

16-subarray Slide

Sub-array Sub-array Sub-array Sub-array Sub-array Sub-array Sub-array Sub-array

Sub-array Sub-array Sub-array Sub-array Sub-array Sub-array Sub-array Sub-array

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NL1	NL1	NL1	NL1	NL2	NL2	NL2	NL2	NL3	NL3	NL3	NL3
NL4	NL4	NL4	NL4	NL5	NL5	NL5	NL5	NL6	NL6	NL6	NL6
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NL37	NL37	NL37	NL37	NL38	NL38	NL38	NL38	NC	NC	NC	NC
PC1	PC1	PC1	PC1	Blank	Blank	Blank	Blank	M	M	M	M

<u>Controls</u>

NC: negative control (Print Buffer)

PC: positive control (a biotinylated probe)

M: Marker

Lectin List

ID	Lectin	ID	Lectin	ID	Lectin
NL1	AAL	NL14	HAA	NL27	PSA
NL2	AIA	NL15	HHL	NL28	PTA
NL3	BC2L-C	NL16	HPA	NL29	RCA-I
NL4	BPL	NL17	LCA	NL30	RPA
NL5	Calsepa	NL18	LEL	NL31	RSL
NL6	ConA	NL19	LTA	NL32	SBA
NL7	DBA	NL20	MAL-I	NL33	SNA
NL8	DSA	NL21	MAL-II	NL34	STL
NL9	ECL	NL22	Morniga G	NL35	UEA-I
NL10	EEA	NL23	NPA	NL36	VVL
NL11	GNA	NL24	PHA-E	NL37	WFA
NL12	GSI-B4	NL25	PHA-L	NL38	WGA
NL13	GSII	NL26	PNA		

Materials Required

- Arrayed glass slides
- 16 or 8 cassettes
- Glycan Array Blocking Buffer (GABB, Item #10106), add 1% BSA (10 mg/ml) if needed
- Lectin Array Assay Buffer (LAAB, Item #10111), add 1% BSA (10 mg/ml) if needed
- Laser fluorescence scanner (able to scan at the wavelength of your fluorophore)
- Coplin jar
- Adhesive slide cover film
-

Preparation of assay samples:

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

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

Part 2 – Binding Assay

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

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

If your glycoprotein samples are fluorescently labelled, go directly to Part 6 – Final Wash and Dry.

Part 3 – Wash

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

If your glycoprotein sample is biotinylated, go directly to Part 5 – Fluorescent Staining.

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

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

Part 5 – Fluorescent Staining

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

Part 6 – Final Wash and Dry

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

Analysis

Scan the slide in a laser fluorescence scanner at the wavelength of emission for the fluorophore used. Adjust the laser power and PMT to obtain the highest possible signals without any being saturated (saturated positive control signal is okay). Analyze data with microarray analysis software. If there is specific binding the signal intensity should be higher than the background signal (area where there are no printed spots). Fluorescent signal due to specific binding to your 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:

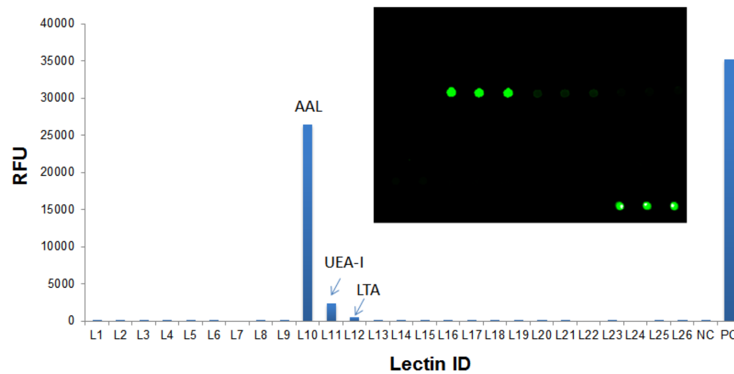
Negative Control (Print Buffer): The negative control should produce a signal close to the intensity of the background. Since there is no binding involved with the negative control, any other signals around the negative control's intensity are

also not binding. Subtracting the negative control's signal from the other binding signals will give more accurate specific binding data.

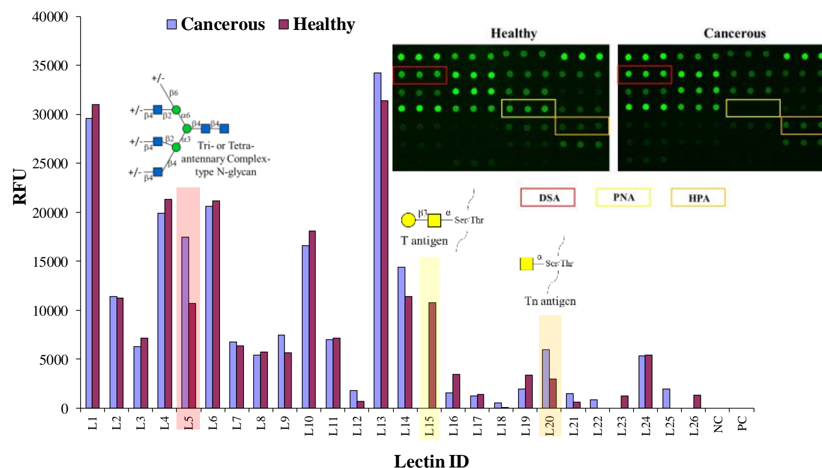
<u>Positive Control (a biotinylated probe):</u> This positive control will bind directly to the fluorescent labelled streptavidin. If your glycoprotein sample is already fluorescently labelled, or in any case where the addition of fluorescent labelled streptavidin to the array was not preformed (Part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

Typical Binding Assay Result from the Lectin Microarray

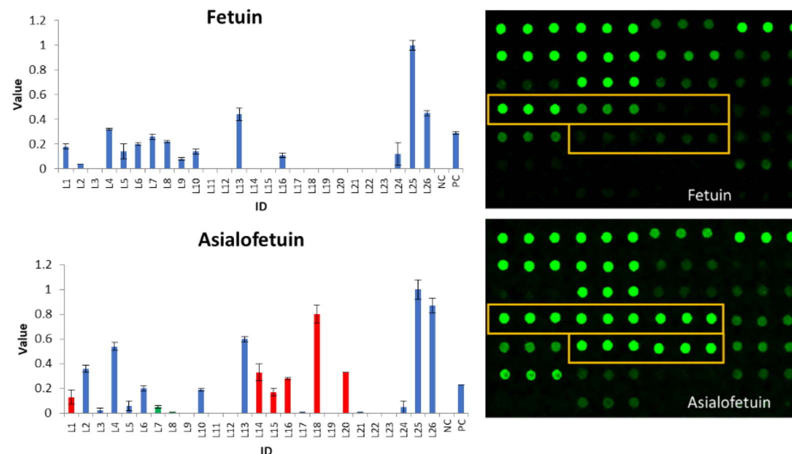
Example 1: A subarray assayed with a biotinylated α-L-fucose target (0.01 μg/ml), followed by streptavidin-Cy3 (1 μg/ml). The array was scanned with GenePix scanner at 500 PMT and 100% laser power at 532nm wavelength. The positive control shows binding as expected. Three lectins, AAL, UEA-I and LTA, show specific binding to the target.



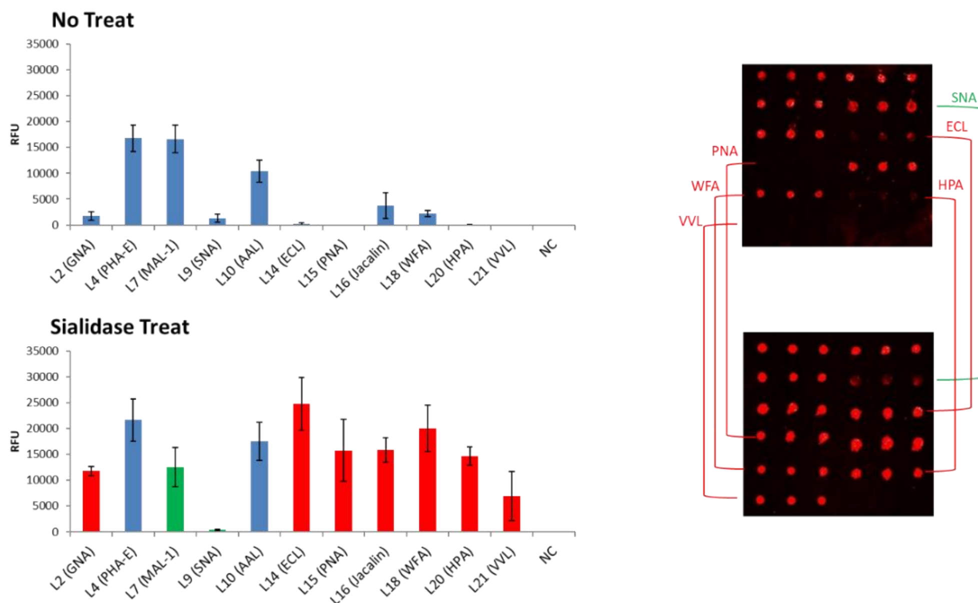
Example 2: Two subarrays assayed with AlexaFluor555-labeled cancerous (breast cancer) and healthy human serum (1:50 dilution). The array was scanned with GenePix scanner at 450 PMT and 100% laser power at 532nm wavelength. Data is normalized by equalizing the sum of the 26 lectin intensities on the cancerous 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.



Example 4: Cell-based Assay. Lectin Microarray was incubated with live cells with or without treatment with sialidase. The sialic acid binding lectins (e.g., MAL-I, SNA) exhibited slightly decreased binding signals on treated cells. However, the signals from galactose- or GalNAc-binding lectins increased greatly.



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