# Lectin Microarray User Manual

Z BIOTECH

Innovative Biochemical Analysis Solutions

**Website:** http://www.zbiotech.com/home.html

**Tel: (720) 285-3587**

**Email:** info@zbiotech.com

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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 40 lectins to include in our second-generation lectin microarray. These 40 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.

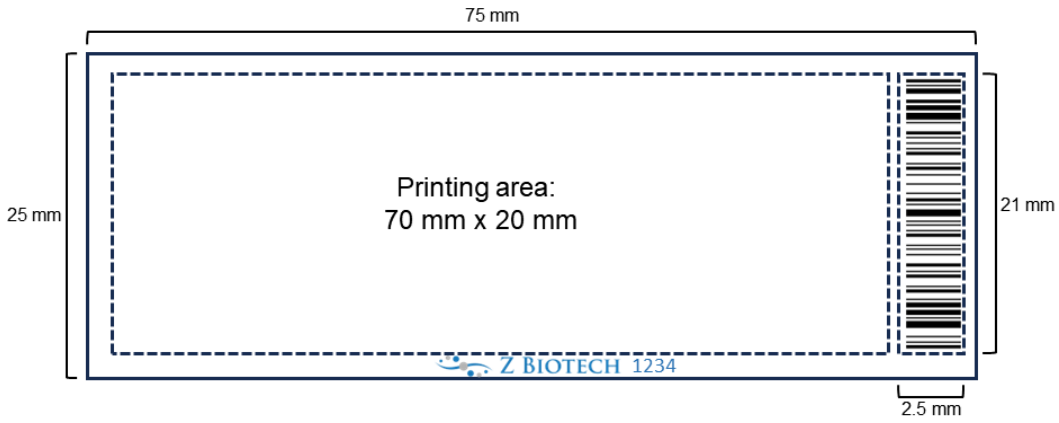
## Handling and Storage

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.

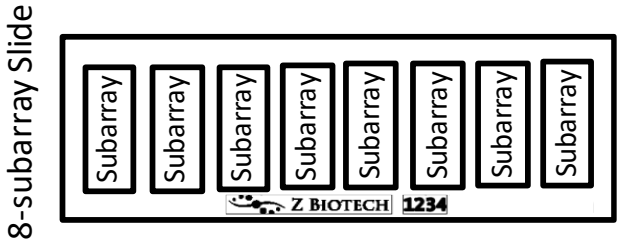
Allow the bag of slides to equilibrate to room temperature at least 20 minutes before opening. After opening, re-seal any unused slides in the moisture barrier bag with a desiccant inside and refreeze.

## 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

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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 |
| NL26 | NL26 | NL26 | NL26 | NL26 | NL26 | NL27 | NL27 | NL27 | NL27 | NL27 | NL27 | NL28 | NL28 | NL28 | NL28 | NL28 | NL28 | NL29 | NL29 | NL29 | NL29 | NL29 | NL29 | NL30 | NL30 | NL30 | NL30 | NL30 | NL30 |
| NL31 | NL31 | NL31 | NL31 | NL31 | NL31 | NL32 | NL32 | NL32 | NL32 | NL32 | NL32 | NL33 | NL33 | NL33 | NL33 | NL33 | NL33 | NL34 | NL34 | NL34 | NL34 | NL34 | NL34 | NL35 | NL35 | NL35 | NL35 | NL35 | NL35 |
| NL36 | NL36 | NL36 | NL36 | NL36 | NL36 | NL37 | NL37 | NL37 | NL37 | NL37 | NL37 | NL38 | NL38 | NL38 | NL38 | NL38 | NL38 | NL39 | NL39 | NL39 | NL39 | NL39 | NL39 | NL40 | NL40 | NL40 | NL40 | NL40 | NL40 |
| NC | NC | NC | NC | NC | NC | PC1 | PC1 | PC1 | PC1 | PC1 | PC1 | PC2 | PC2 | PC2 | PC2 | PC2 | PC2 | PC3 | PC3 | PC3 | PC3 | PC3 | PC3 | PC4 | PC4 | PC4 | PC4 | PC4 | PC4 |
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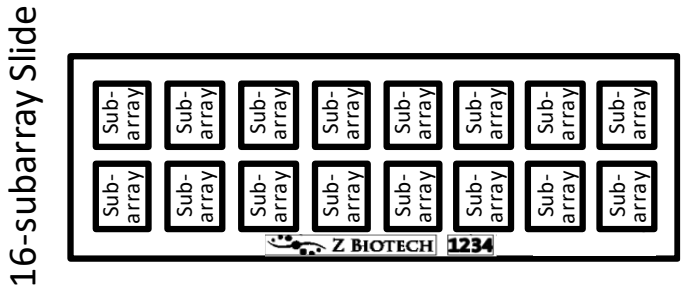
## 16-subarray Array Layout

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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 | NL7 | NL7 | NL7 | NL7 | NL8 | NL8 | NL8 | NL8 |
| NL9 | NL9 | NL9 | NL9 | NL10 | NL10 | NL10 | NL10 | NL11 | NL11 | NL11 | NL11 | NL12 | NL12 | NL12 | NL12 |
| NL13 | NL13 | NL13 | NL13 | NL14 | NL14 | NL14 | NL14 | NL15 | NL15 | NL15 | NL15 | NL16 | NL16 | NL16 | NL16 |
| NL17 | NL17 | NL17 | NL17 | NL18 | NL18 | NL18 | NL18 | NL19 | NL19 | NL19 | NL19 | NL20 | NL20 | NL20 | NL20 |
| NL21 | NL21 | NL21 | NL21 | NL22 | NL22 | NL22 | NL22 | NL23 | NL23 | NL23 | NL23 | NL24 | NL24 | NL24 | NL24 |
| NL25 | NL25 | NL25 | NL25 | NL26 | NL26 | NL26 | NL26 | NL27 | NL27 | NL27 | NL27 | NL28 | NL28 | NL28 | NL28 |
| NL29 | NL29 | NL29 | NL29 | NL30 | NL30 | NL30 | NL30 | NL31 | NL31 | NL31 | NL31 | NL32 | NL32 | NL32 | NL32 |
| NL33 | NL33 | NL33 | NL33 | NL34 | NL34 | NL34 | NL34 | NL35 | NL35 | NL35 | NL35 | NL36 | NL36 | NL36 | NL36 |
| NL37 | NL37 | NL37 | NL37 | NL38 | NL38 | NL38 | NL38 | NL39 | NL39 | NL39 | NL39 | NL40 | NL40 | NL40 | NL40 |
| NC | NC | NC | NC | PC1 | PC1 | PC1 | PC1 | PC2 | PC2 | PC2 | PC2 | PC3 | PC3 | PC3 | PC3 |
| PC4 | PC4 | PC4 | PC4 | Blank | Blank | Blank | Blank | Blank | Blank | Blank | Blank | M | M | M | M |

## Controls

NC: Negative control, Print Buffer
PC1: Positive control 1, Biotinylated PEG (0.01 mg/mL)
PC2: Positive control 2, Human IgG (0.1 mg/mL)
PC3: Positive control 3, Mouse IgG (0.1 mg/mL)

PC4: Positive control 4, Rabbit IgG (0.1 mg/mL)
Marker: Anti-human IgG, Cy3 (0.01 mg/mL) and anti-Human IgG, Alexa647 (0.01 mg/mL)

**Lectin List**

| ID | Lectin | ID | Lectin | ID | Lectin | ID | Lectin |
|---|---|---|---|---|---|---|---|
| NL1 | AAL | NL11 | GSI-B4 | NL21 | Morniga G | NL31 | SBA |
| NL2 | AIA | NL12 | GSII | NL22 | NPA | NL32 | SNA |
| NL3 | BC2L-C | NL13 | HAA | NL23 | PHA-E | NL33 | STL |
| NL4 | BPL | NL14 | HHL | NL24 | PHA-L | NL34 | UEA-I |
| NL5 | Calsepa | NL15 | HPA | NL25 | PNA | NL35 | VVL |
| NL6 | ConA | NL16 | LCA | NL26 | PSA | NL36 | WFA |
| NL7 | DBA | NL17 | LEL | NL27 | PTA | NL37 | WGA |
| NL8 | DSA | NL18 | LTA | NL28 | RCA-I | NL38 | RPL-Sia2 |
| NL9 | ECL | NL19 | MAL-I | NL29 | RPA | NL39 | RPL-Man2 |
| NL10 | GNA | NL20 | MAL-II | NL30 | RSL | NL40 | RPL-αGal |

**Materials Required**

- Arrayed glass slides
- 16 or 8 cassettes
- Glycan Array Blocking Buffer (GABB, Item #10106) 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 (if needed)

*Our array substrates have been coated with blocking reagents. In most cases, the blocking step is not required.*

*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.

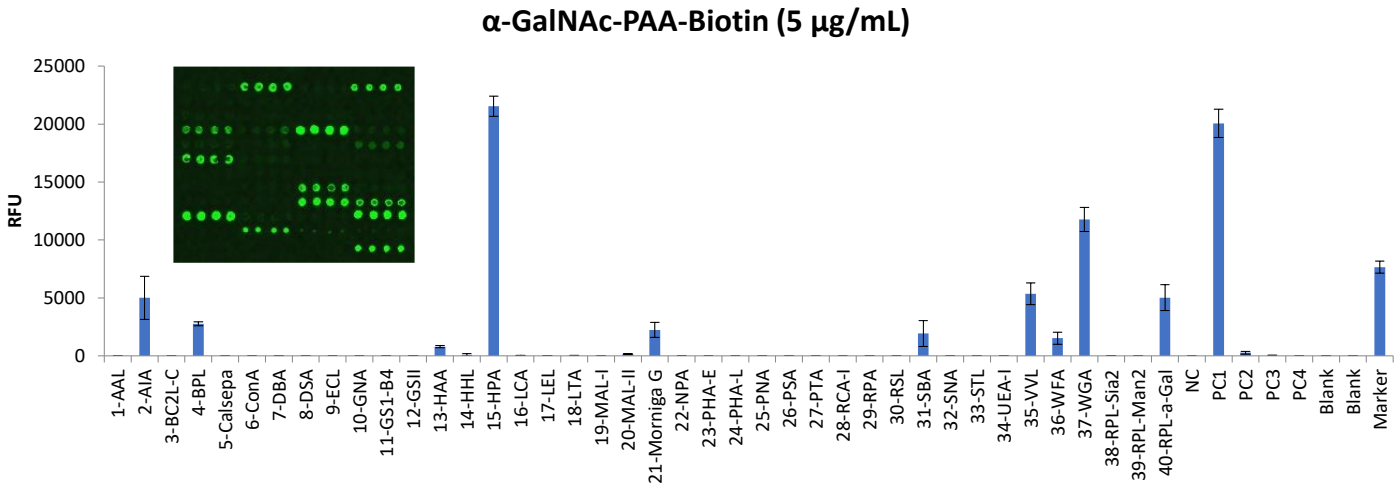
Interpretation of Control Signals:

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.

Positive Control (a biotinylated probe): 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**

A subarray of the Lectin Microarray slide was assayed with a biotinylated α-GalNAc-PAA target (5 µg/ml), followed by streptavidin-Cy3. The array was scanned with InnoScan 710 microarray scanner at 2 PMT and low laser power at 532nm wavelength. There is no non-specific binding for the negative control spots. Positive control 1 and the marker show binding as expected. The lectins (e.g., HPA, VVL) which have binding specificity to α-GalNAc glycans showed expected binding signals.



**Troubleshooting**

| Condition | Possible Causes | Potential Solutions |
|---|---|---|
| High Background | • Concentration of protein samples is too high<br>• Concentration of fluorescent samples is too high<br>• Arrays are not thoroughly washed.<br>• Slide drying out during assay<br>• 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.<br>• Apply longer times for washing steps and use a higher shaking rate<br>• Make sure wash buffer and sample is completely removed before the next step<br>• Make sure adhesive film fully seals the wells to avoid evaporation<br>• Centrifuge the samples prior to assay to avoid adding irrelevant particles. Make sure buffers are filtered.<br>• If you think that the protein is aggregating during incubation, try shaking at a higher speed |
| Signal Variation | • Slide drying out during assay<br>• Binding samples are not equally distributed in the wells<br>• Glycan-binding protein aggregation during incubation<br>• Bubbles during incubation | • Make sure wells are sealed to prevent evaporation during incubation<br>• Apply a larger volume of sample to each well to ensure equal distribution<br>• Use a higher shaking rate during incubation<br>• 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<br>• Sample contamination | • Make sure to use sterilized pipette tips and tubes used for sample application and preparation<br>• Ensure cassette is pressed firmly to the slide so that there are no gaps to allow leaking between wells<br>• Be careful not to cross contaminate samples when applying to the wells, even during wash steps |