MUC1 Glycopeptide Array 2

User Manual

Innovative Biochemical Analysis Solutions

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Introduction

Mucin 1 (MUC1) is a transmembrane glycoprotein, and it is one of several mucin proteins that make up mucus. The extracellular domain of MUC1 consists of a variable number of 20-amino-acid tandem repeats, and these repeats are highly O-glycosylated. α-N-acetylgalactosamine (GalNAc, Tn) is attached to the hydroxyl group of threonine (Thr) and serine (Ser) of the tandem repeats and further extended with galactose (Gal), N-acetylglucosamine (GlcNAc), or GalNAc to form eight different core structures. These structures can be further modified by sialylation, sulfation, acetylation, fucosylation, and polylactosamine extension.

MUC1 is expressed on the surface of normal epithelial cells. However, changes in glycosylation patterns occur in various pathological conditions. For example, aberrant glycosylation of MUC1 has prevented cancer cells from forming core-2-based glycans. Therefore, cancer-associated MUC1 is hypoglycosylated with short carbohydrate structures such as Tn (GalNAc-Ser or -Thr) and STn (Neu5Ac-6-GalNAc-Thr). This unique feature makes MUC1 an ideal cancer-specific antigen for therapeutics development.

ZBiotech has developed a robust microarray platform that allows researchers to screen a large MUC1 glycopeptide library. The current MUC1 glycopeptide array 2 includes 124 MUC1 glycopeptides and 1 unglycosylated control peptide. Each glycopeptide is 23-mer in length and modified with 3-ST, 6-ST, Core 2, and/or diST. This microarray allows researchers to identify, profile, and compare specific antibody responses and detect and validate protein interactions with various MUC1 glycoforms. This array is highly sensitive with low background. The MUC1 glycopeptide array can be customized to meet individual client needs. Assay services are available upon request. We also provide services to synthesize various MUC1 glycopeptides.

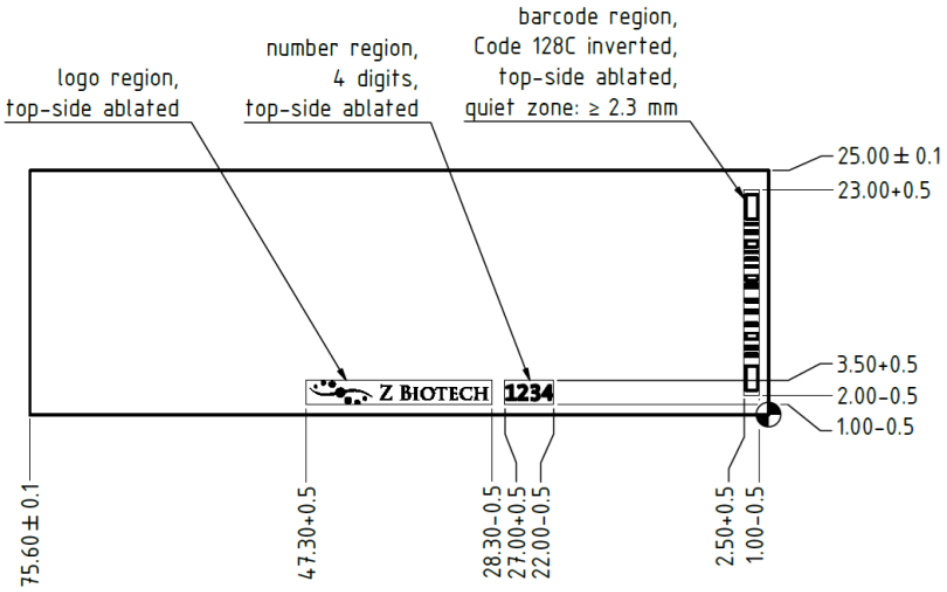
Handling and Storage

Store the bag of slides and any buffers in a 4°C refrigerator if they are to be assayed within 24 hours 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

MUC1 Glycopeptide Array 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.


logo region,
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number region,
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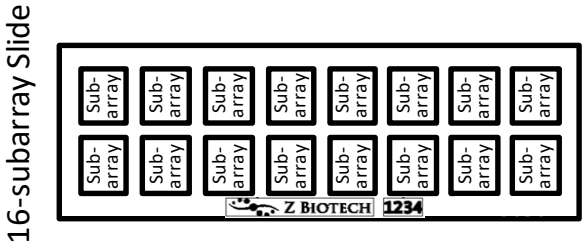
top-side ablated
barcode region,
Code 128C inverted,
top-side ablated,
quiet zone: ≥ 2.3 mm
25.00 ± 0.1
23.00+0.5
3.50+0.5
2.00-0.5
1.00-0.5
Z BIOTECH
1234
75.60 ± 0.1
47.30+0.5
28.30-0.5
27.00+0.5
22.00-0.5
2.50+0.5
1.00-0.5

Array Map (16-subarray slides)



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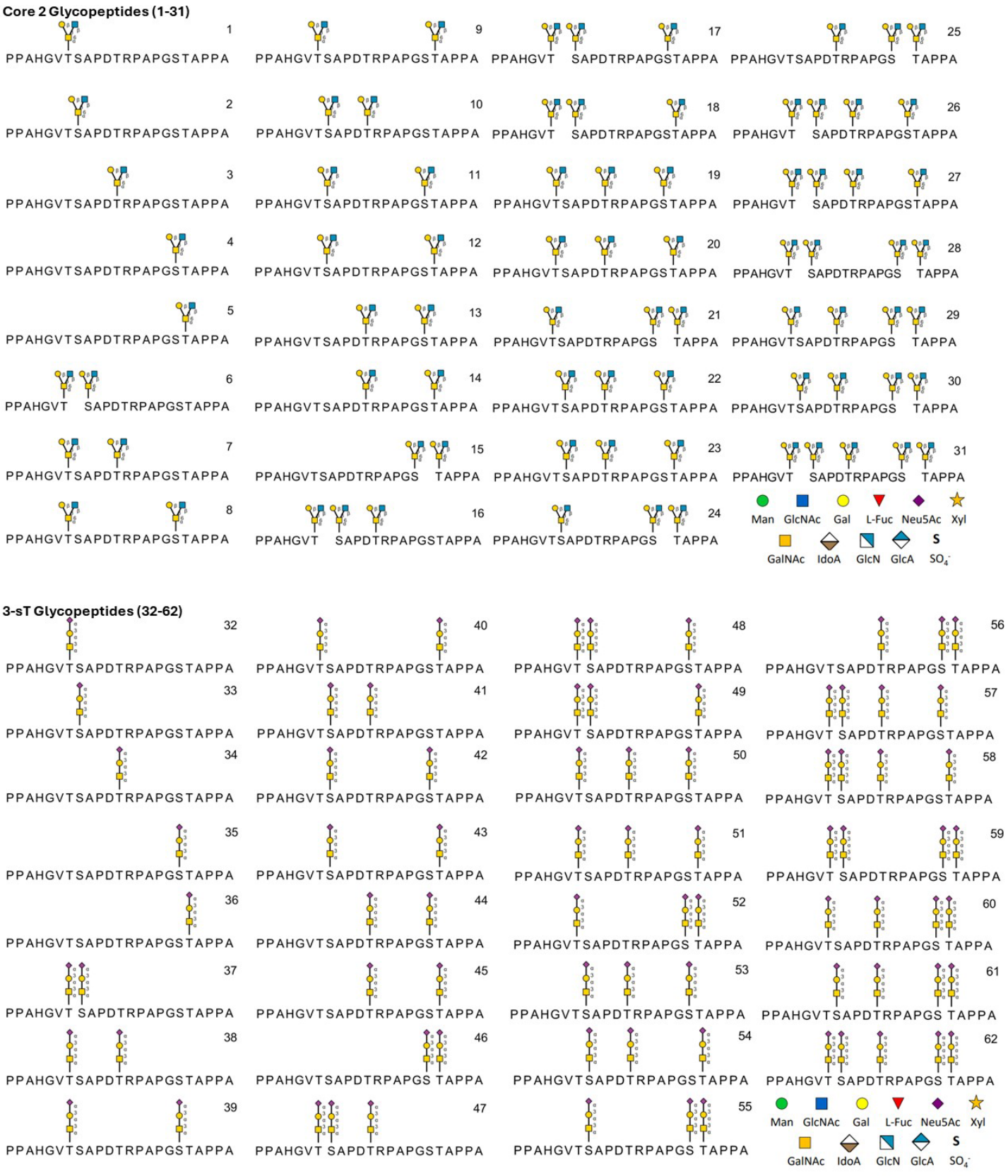
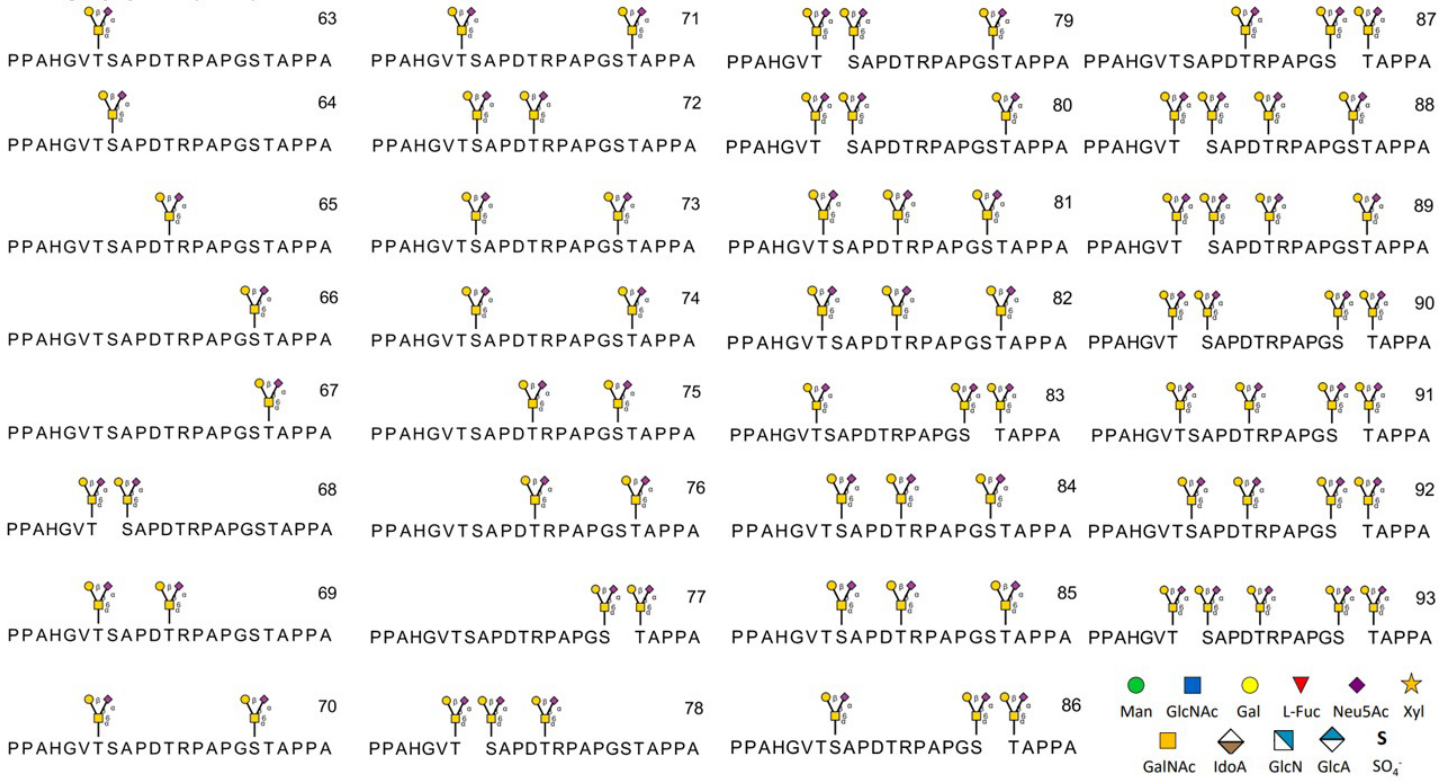
120	120	120	121	121	121	122	122	122	123	123	123	124	124	124	125	125	125	Blank	Blank	Blank
Blank	Blank	Blank	NC	NC	NC	PC1	PC1	PC1	PC2	PC2	PC2	PC3	PC3	PC3	PC4	PC4	PC4	M	M	M

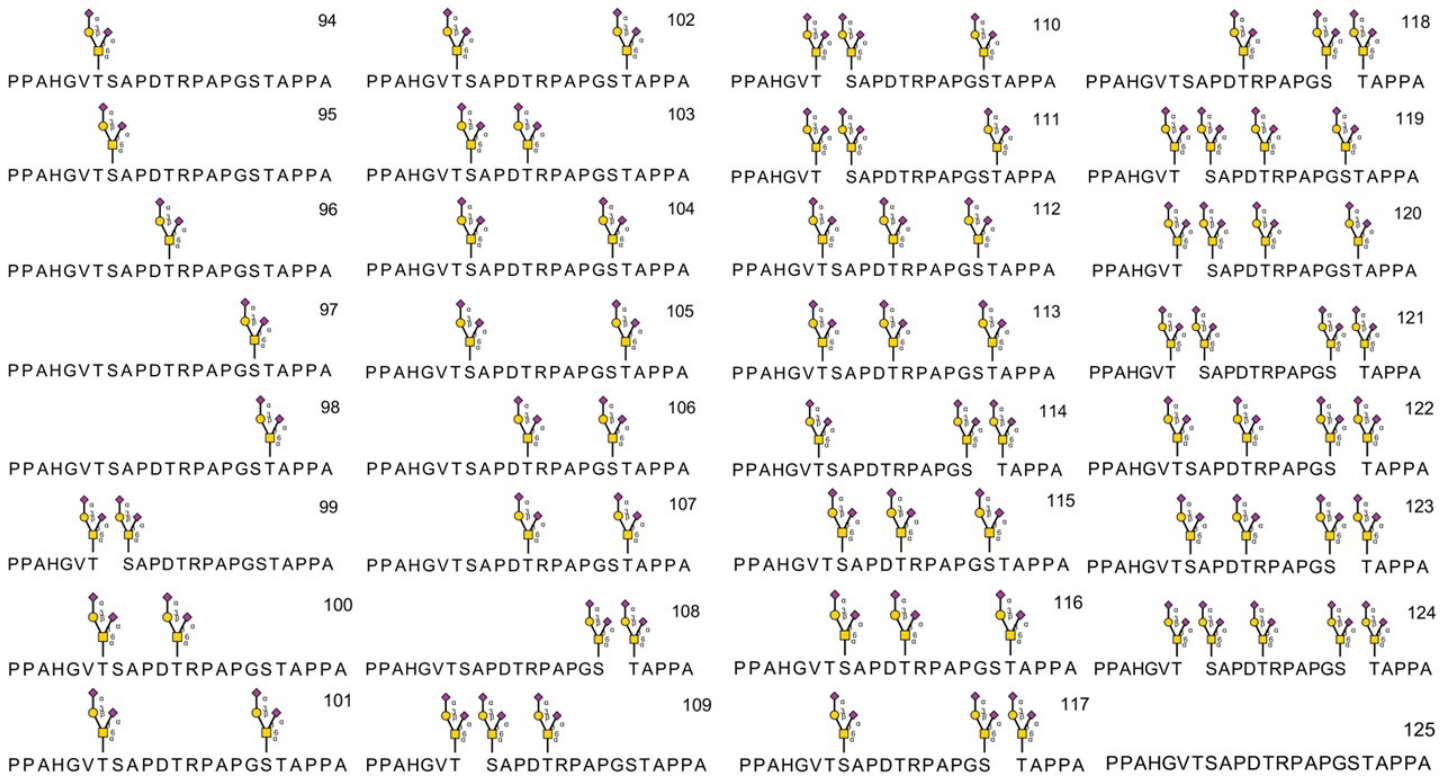
Structures of the MUC1 Glycopeptides



6-sT Glycopeptides (63-93)



di-sT Glycopeptides (94-124) and non-glycosylated peptide (125)



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)

Materials Required

- Arrayed glass slides
- 16 or 8 cassettes
- Glycan Array Blocking Buffer (GABB, Item #10106), add 1% BSA (10 mg/ml) if needed
- Glycan Array Assay Buffer (GAAB, Item #10107), 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 glycan-binding protein samples or secondary antibodies of interest in a centrifuge tube by diluting with the Glycan Array Assay Buffer. We recommend a range of 50 µg/ml to 0.1 µg/ml concentration for protein samples, although some experimentation may be required to establish the concentration that will provide the highest binding signals with the lowest background fluorescence. This is often accomplished by applying a different dilution of samples to different wells of the array. For the fluorescently labelled streptavidin we recommend a concentration of 1 µg/mL. Calculate the volume of sample needed depending on how many slides and subarrays are to be assayed. **We recommend using 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 for every step of the assay.** If necessary, the assay can be done successfully with a minimal volume of 60 µL per well for 16 subarray cassettes and 80 µL for 8 subarray cassettes. We caution that 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 glycan binding protein sample of interest is bacteria or cells, centrifuge samples briefly to avoid adding irrelevant 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 GAAB to each well and shaking the array at 80 rpm for 5 min. Remove the buffer and repeat.
4. Immediately apply the glycan binding protein 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 glycan-binding protein 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 GAAB 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 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 third GAAB 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 GAAB 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 GAAB.
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 GAAB. 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 sample of interest should be both dose-dependent with your sample dilution (unless the sample concentration range is too high and glycan binding is saturated) 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 Controls (Print Buffer): The negative controls should produce little to no signal. Since there is no binding involved with the negative control, any other signals around the negative control's intensity are also not binding.

Marker: The array marker should show a strong fluorescence signal regardless of the assay. It is there primarily to aid with orientation of the array map during analysis.

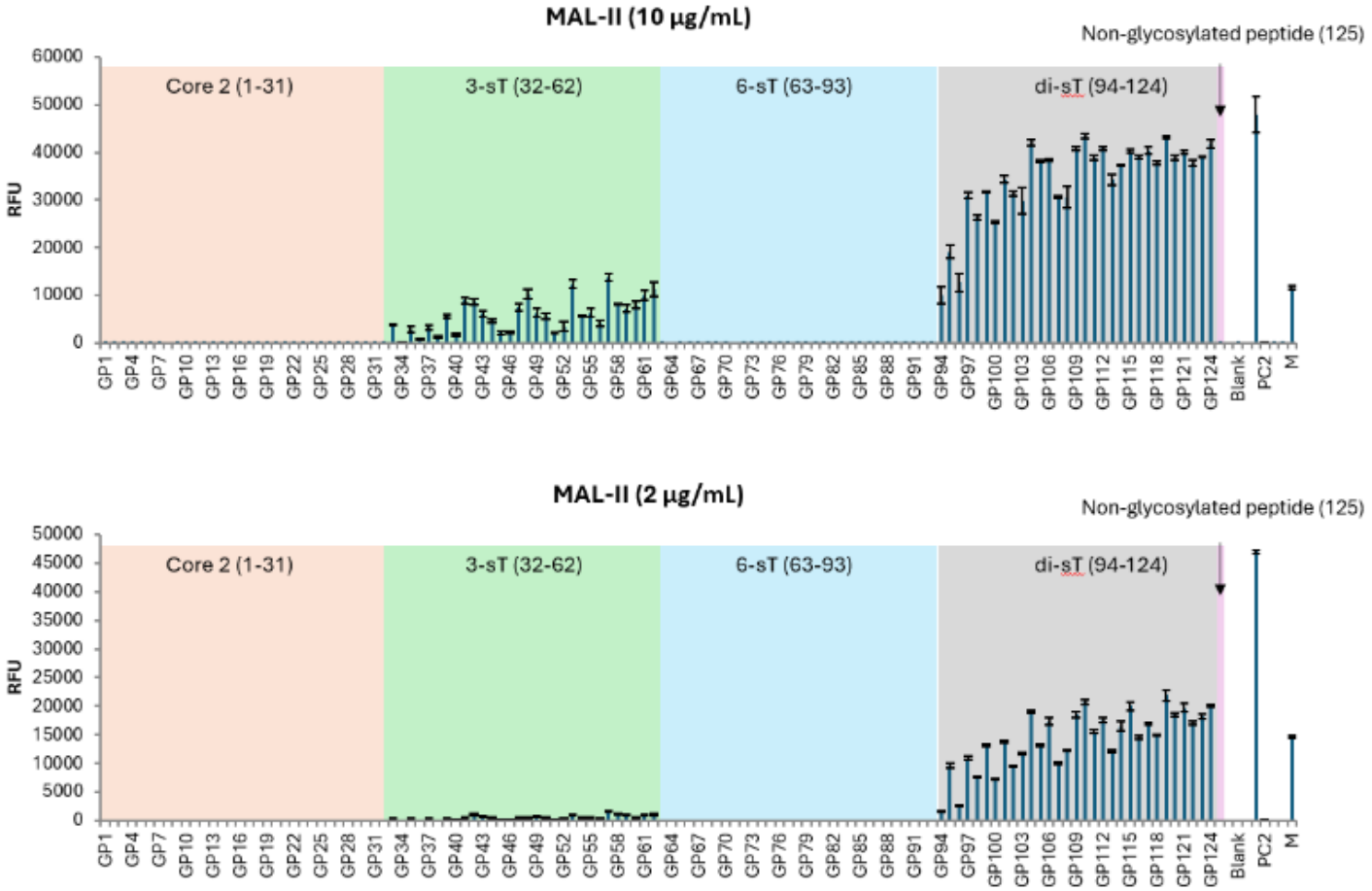
Biotinylated PEG (PC1): This positive control will bind directly to the fluorescent labelled streptavidin. If your glycan-binding protein sample is already fluorescently labelled, or in any case where the addition of fluorescent labelled streptavidin to the array was not preformed (Part 5 – Fluorescent staining) this positive control will not be reactive.

IgG (PC2, PC3, PC4): IgG is an antibody found in blood that is a primary component of humoral immunity. If the glycan-binding or secondary antibody sample is an anti-IgG from human, rabbit, or mouse it should bind to the respective IgG control.

Typical Binding Assay Result from the MUC1 Glycopeptide Array

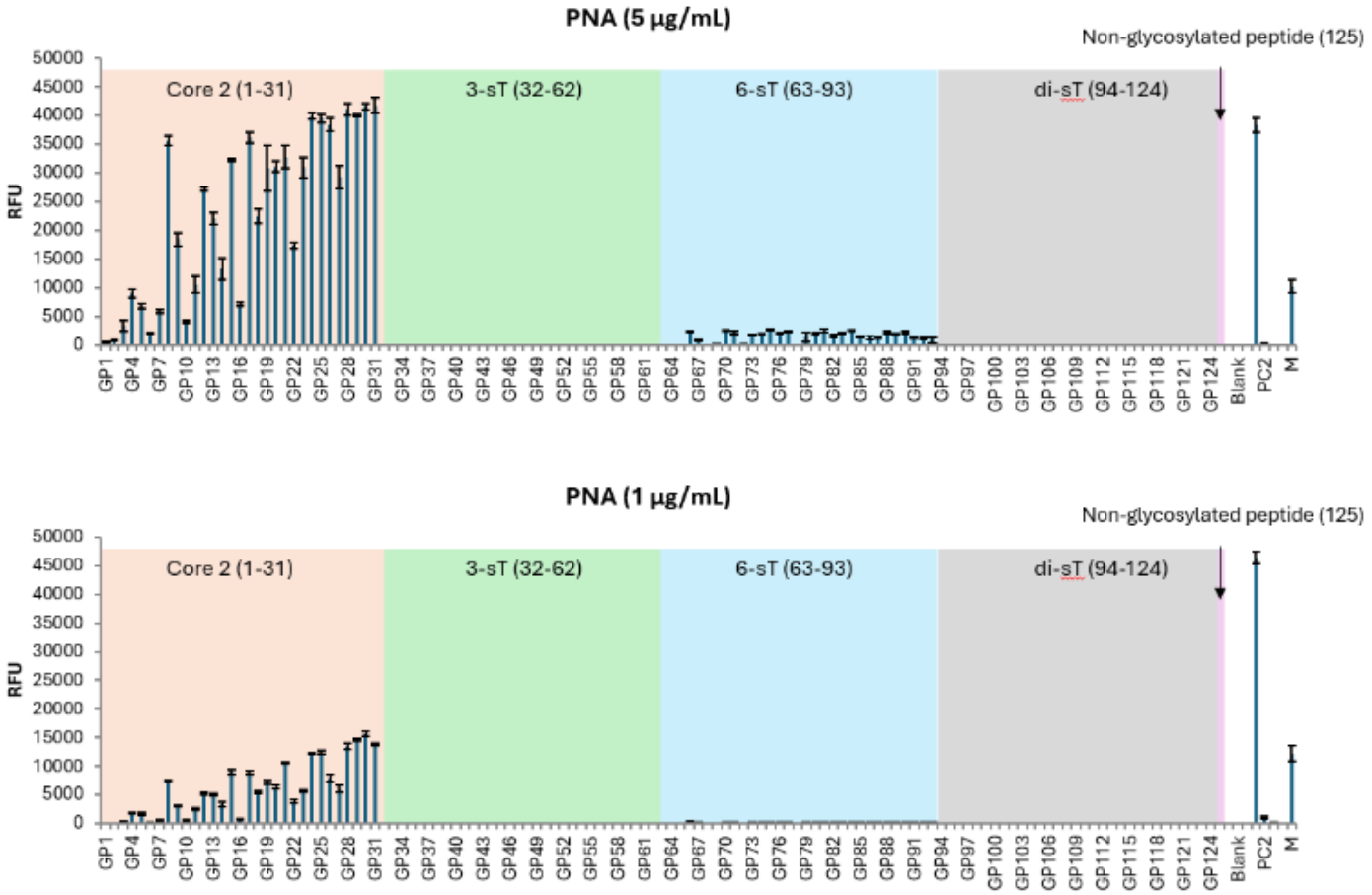
MAL-II (Maackia amurensis lectin II) primarily binds to di-sT with weaker binding to 3-sT.

The MUC1 Glycopeptide Array 2 was utilized to assess the binding specificity of biotinylated MAL-II lectin at concentrations of 10 or 2 μg/mL. After incubating with MAL-II, the array was washed and treated with Cy3-conjugated streptavidin for detection. Scanning was performed using a microarray scanner at a wavelength of 532 nm. The positive control exhibited expected binding signals. MAL-II showed specific binding to MUC1 glycopeptides modified with di-sT and 3-sT.



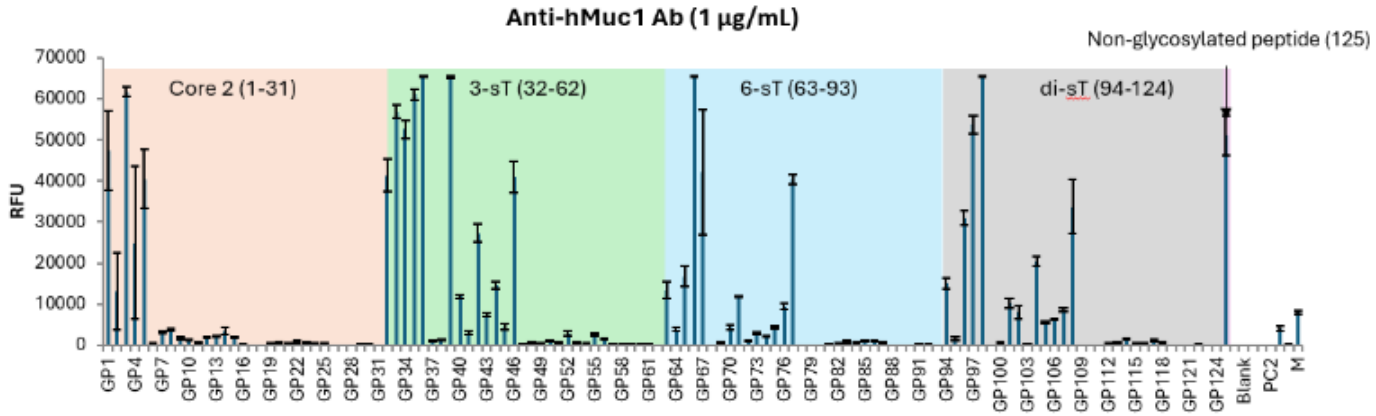
PNA (Peanut Agglutinin) primarily binds to Core 2 with weaker binding to 6-sT.

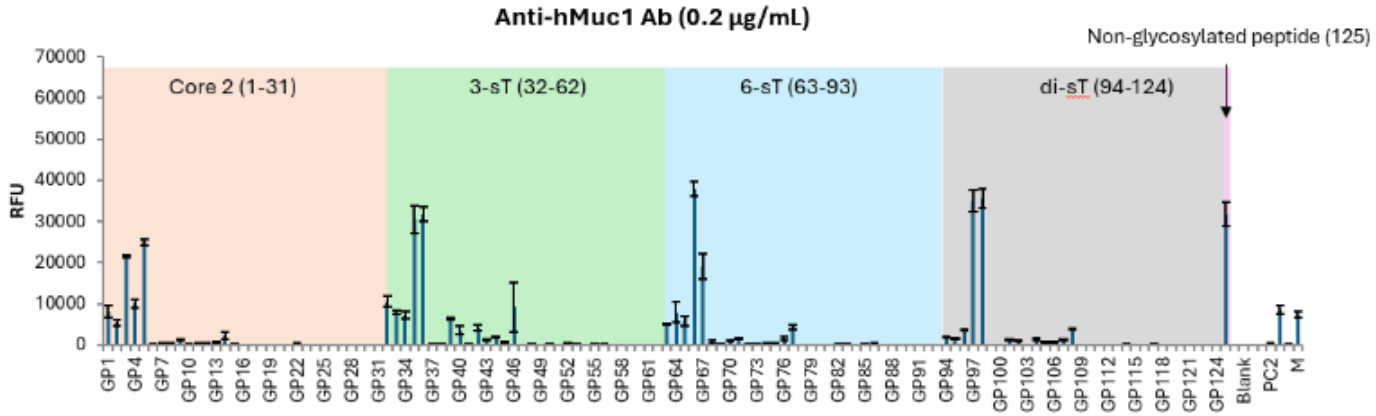
The MUC1 Glycopeptide Array 2 was utilized to assess the binding specificity of biotinylated PNA at concentrations of 5 or 1 µg/mL. After incubating with PNA, the array was washed and treated with Cy3-conjugated streptavidin for detection. Scanning was performed using a microarray scanner at a wavelength of 532 nm. The positive control exhibited expected binding signals. PNA showed specific binding to MUC1 glycopeptides modified with Core2 and 6-sT.



The Anti-hMUC1 antibody showed differential binding to MUC1 glycopeptides modified with 3-ST, 6-ST, Core 2, and/or diST.

The MUC1 Glycopeptide Array 2 was utilized to assess the binding specificity of the Anti-hMUC1 antibody at concentrations of 1 or 0.2 µg/mL. After incubating with the antibody, the array was washed and treated with an Anti-Mouse IgG – AF555 antibody for detection. Scanning was performed using a microarray scanner at a wavelength of 532 nm. The positive control exhibited expected binding signals. Specific modifications with 3-ST, 6-ST, Core 2, or diST moderately increased the binding of the antibody, while most other modifications significantly reduced the binding.


Anti-hMuc1 Ab (1 µg/mL)
Non-glycosylated peptide (125)
Core 2 (1-31)
3-sT (32-62)
6-sT (63-93)
di-sT (94-124)
RFU
70000
60000
50000
40000
30000
20000
10000
0
GP1 GP4 GP7 GP10 GP13 GP16 GP19 GP22 GP25 GP28 GP31 GP34 GP37 GP40 GP43 GP46 GP49 GP52 GP55 GP58 GP61 GP64 GP67 GP70 GP73 GP76 GP79 GP82 GP85 GP88 GP91 GP94 GP97 GP100 GP103 GP106 GP109 GP112 GP115 GP118 GP121 GP124 Blank PC2 M


Anti-hMuc1 Ab (0.2 µg/mL)
Non-glycosylated peptide (125)
Core 2 (1-31)
3-sT (32-62)
6-sT (63-93)
di-sT (94-124)
RFU
70000
60000
50000
40000
30000
20000
10000
0
GP1 GP4 GP7 GP10 GP13 GP16 GP19 GP22 GP25 GP28 GP31 GP34 GP37 GP40 GP43 GP46 GP49 GP52 GP55 GP58 GP61 GP64 GP67 GP70 GP73 GP76 GP79 GP82 GP85 GP88 GP91 GP94 GP97 GP100 GP103 GP106 GP109 GP112 GP115 GP118 GP121 GP124 Blank PC2 M

Troubleshooting

Condition	Possible Causes	Potential Solutions
High Background	• Concentration of glycan-binding 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. • 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 • 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