100 N-Glycan Array User Manual

Z BIOTECH

Innovative Biochemical Analysis Solutions

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

Tel: (720) 859-3551

Email: info@zbiotech.com

For Research Use Only

Copyright 2018, Z Biotech, LLC. All Rights Reserved.

Table of Contents

Introduction

Glycans attached to cell membranes and other types of proteins are the primary determinants for binding activity and consecutive cellular function. The study of the function and characteristics of these sugars is a fundamental part of immunology research and is applicable in understanding a variety of intercellular interactions. New technologies such as microarray for glycan-binding applications allow researchers to investigate and reveal new information about this wide and developing field of glycoscience.

Z Biotech's 100 N-Glycan Array is used as a general test to help researchers determine binding characteristics of antibodies, proteins, bacteria, cell cultures, or other potential biological samples to an array of 100 fundamental N-glycans. Our microarray slides are especially coated to be capable of immobilizing natural N-glycans with close-ring structure at their reducing end (GlcNAc). N-linked oligosaccharides in particular play a major role in intercellular interactions and immune cell functions, making them often preferred glycan candidates for the study of carbohydrate-binding-vaccines such as HIV Broadly Neutralizing Antibodies. The fundamental N-glycan structures provided in this array can provide understanding of basal binding determinants for antibodies or other proteins of interest. This manual is provided as a comprehensive guide to help the researcher acquire clear results from the assay. Please read through carefully before starting your experiment.

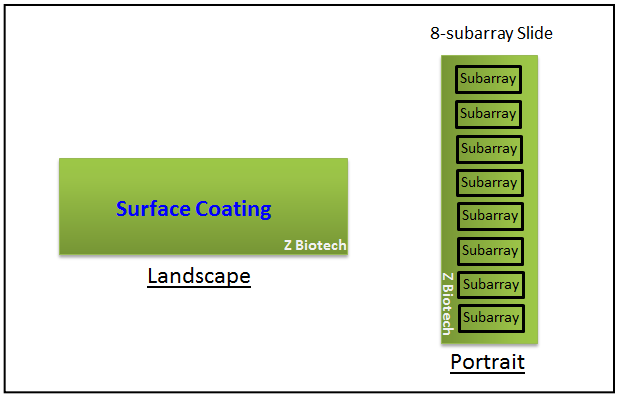
Handling and Storage

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. Handle the slides in a clean, dust free environment. Wear gloves and hold the slides on the edges. When adding sample do not touch the pipette tip to the array surface. When removing sample gently touch the pipette tip at the corner of the well of the cassette and tip the slide so that the sample pools to that corner. Avoid contact with the surface of the slides.

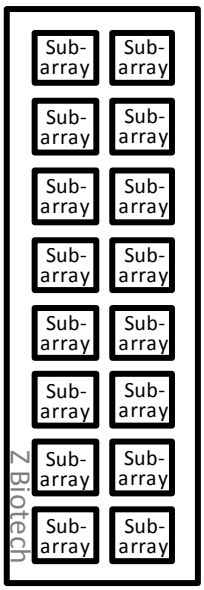
Array Map/Schematic

100 N-Glycan Array slides have 8 or 16 subarrays depending on the customer's request. 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 (the "marker" spots will be on the lower



right of each array when looking at the slide via the portrait orientation). Dimensions and array map are shown on the following pages.

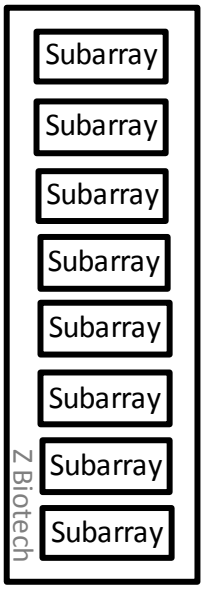
16-subarray Slide



Array Map:

N000	N000	N001	N001	N002	N002	N003	N003	N004	N004	N005	N005	N010	N010	N011	N011
N012	N012	N013	N013	N014	N014	N015	N015	N020	N020	N021	N021	N022	N022	N023	N023
N024	N024	N025	N025	N030	N030	N031	N031	N032	N032	N033	N033	N034	N034	N035	N035
N040	N040	N041	N041	N042	N042	N043	N043	N044	N044	N045	N045	N050	N050	N051	N051
N052	N052	N053	N053	N054	N054	N055	N055	N110	N110	N111	N111	N112	N112	N113	N113
N114	N114	N115	N115	N122	N122	N123	N123	N124	N124	N125	N125	N133	N133	N134	N134
N135	N135	N144	N144	N155	N155	N210	N210	N211	N211	N212	N212	N213	N213	N214	N214
N215	N215	N222	N222	N223	N223	N224	N224	N225	N225	N233	N233	N234	N234	N244	N244
N255	N255	N6000	N6000	N6030	N6030	N6111	N6111	N6112	N6112	N6113	N6113	N6122	N6122	N6123	N6123
N6144	N6144	N6211	N6211	N6212	N6212	N6213	N6213	N6222	N6222	N6223	N6223	N6244	N62244	N3001	N3001
N3004	N3004	Man-1	Man-1	Man-2A	Man-2A	Man-2B	Man-2B	Man-3	Man-3	Man-5	Man-5	Man-6	Man-6	Man-6D2	Man-6D2
Man-7	Man-7	Man-8	Man-8	Man-9	Man-9	N002G	N002G	N003G	N003G	N012G	N012G	N013G	N013G	N015G	N015G
N022G	N022G	N025G	N025G	N112G	N112G	N113G	N113G	NC	NC	PC1	PC1	PC2	PC2	PC3	PC3
PC4	PC4													Marker	Marker

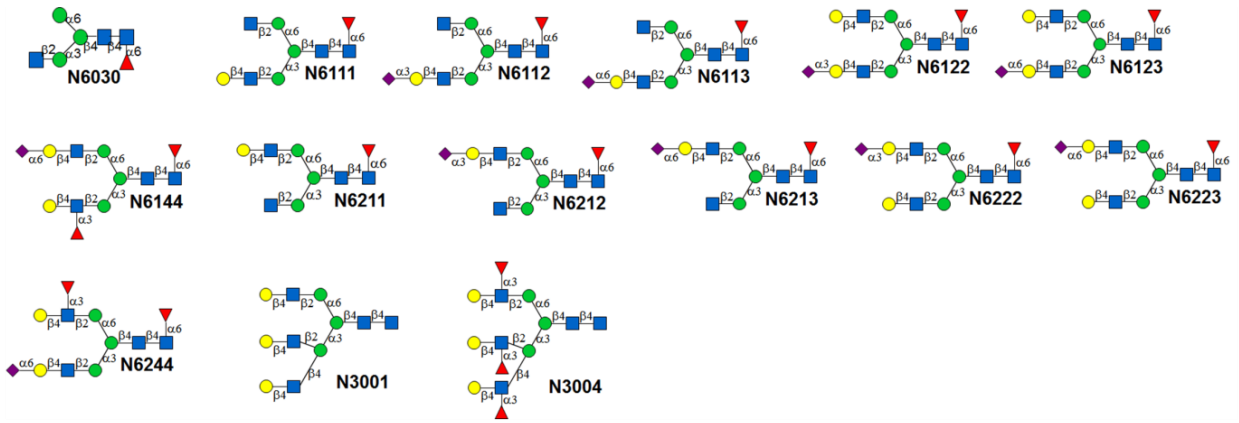
8-subarray Slide

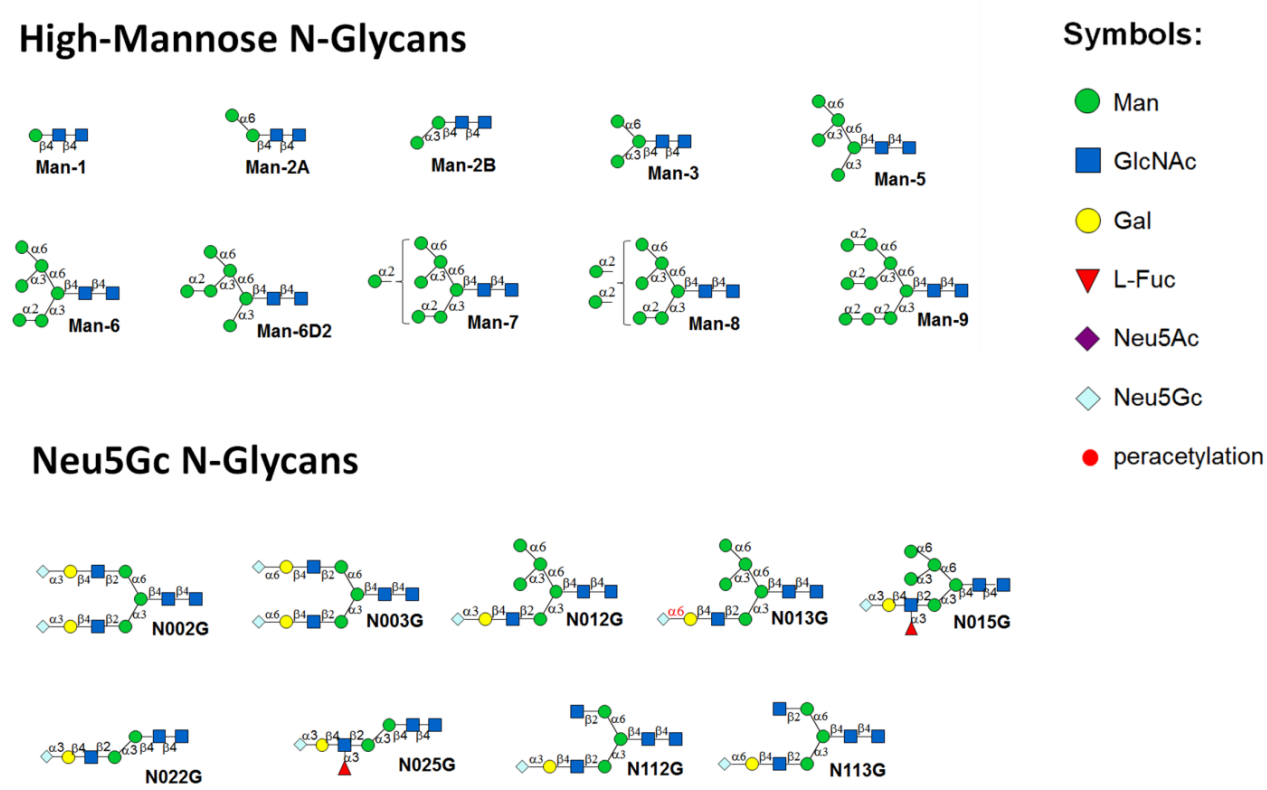


Array Map:

N000	N000	N000	N001	N001	N001	N002	N002	N002	N003	N003	N003	N004	N004	N004	N005	N005	N005	Man-1	Man-1	Man-1	N002G	N002G	N002G	N003G	N003G	N003G
N010	N010	N010	N011	N011	N011	N012	N012	N012	N013	N013	N013	N014	N014	N014	N015	N015	N015	Man-2A	Man-2A	Man-2A	N012G	N012G	N012G	N013G	N013G	N013G
N020	N020	N020	N021	N021	N021	N022	N022	N022	N023	N023	N023	N024	N024	N024	N025	N025	N025	Man-2B	Man-2B	Man-2B	N015G	N015G	N015G	N022G	N022G	N022G
N030	N030	N030	N031	N031	N031	N032	N032	N032	N033	N033	N033	N034	N034	N034	N035	N035	N035	Man-3	Man-3	Man-3	N025G	N025G	N025G	N112G	N112G	N112G
N040	N040	N040	N041	N041	N041	N042	N042	N042	N043	N043	N043	N044	N044	N044	N045	N045	N045	Man-5	Man-5	Man-5	N113G	N113G	N113G			
N050	N050	N050	N051	N051	N051	N052	N052	N052	N053	N053	N053	N054	N054	N054	N055	N055	N055	Man-6	Man-6	Man-6						
N110	N110	N110	N111	N111	N111	N112	N112	N112	N113	N113	N113	N114	N114	N114	N115	N115	N115	Man-6D2	Man-6D2	Man-6D2		NC	NC	NC		
N122	N122	N122	N123	N123	N123	N124	N124	N124	N125	N125	N125	N133	N133	N133	N134	N134	N134	Man-7	Man-7	Man-7		PC1	PC1	PC1		
N135	N135	N135	N144	N144	N144	N155	N155	N155	N210	N210	N210	N211	N211	N211	N212	N212	N212	Man-8	Man-8	Man-8		PC2	PC2	PC2		
N213	N213	N213	N214	N214	N214	N215	N215	N215	N222	N222	N222	N223	N223	N223	N224	N224	N224	Man-9	Man-9	Man-9		PC3	PC3	PC3		
N225	N225	N225	N233	N233	N233	N234	N234	N234	N244	N244	N244	N255	N255	N255	N6000	N6000	N6000					PC4	PC4	PC4		
N6030	N6030	N6030	N6111	N6111	N6111	N6112	N6112	N6112	N6113	N6113	N6113	N6122	N6122	N6122	N6123	N6123	N6123									
N6144	N6144	N6144	N6211	N6211	N6211	N6212	N6212	N6212	N6213	N6213	N6213	N6222	N6222	N6222	N6223	N6223	N6223									
N6244	N6244	N6244	N3001	N3001	N3001	N3004	N3004	N3004																	Marker	Marker

100 N-Glycan Identification List:





Controls

NC: Print Buffer

PC1: Biotinylated mannose (0.01 mg/ml)

PC2: Human IgG (0.1 mg/ml)

PC3: Mouse IgG (0.1 mg/ml)

PC4: Rabbit IgG (0.1 mg/ml)

Array Marker: anti-Human IgG, Cy3 (0.01 mg/ml) + anti-Human IgG, Alexa647 (0.01 mg/ml)

Materials Required

- Arrayed glass slide
- 16 or 8 subarray cassette
- Blocking Buffer (Item #10109): 1% BSA in PBST (PBS with 0.05% (v/v) Tween-20, pH 7.4)
- Glycan Array Assay Buffer (Item #10107)

Materials above are included for purchase in our Microarray Kit (http://www.zbiotech.com/products.html)

- Wash Buffer: 20 mM Tris-HCl, 150 mM NaCl, 0.05% Tween 20, pH 7.6
- Glycan-binding protein samples of interest
- Biotinylated secondary antibodies (for sandwich assay format only)
- Fluorescent labelled streptavidin (for biotin labelled glycan-binding samples)
- Sterile de-ionized water
- Orbital shaker
- Laser fluorescence scanner (able to scan at the wavelength of your fluorophore)
- Centrifuge
- Pipettors and sterile pipette tips
- Sterile centrifuge tubes
- Coplin jar or 250mL beaker
- Adhesive slide cover film
- Aluminum foil

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 in order 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 80 μL for 8 subarray. 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. Allow the arrayed slides to equilibrate to room temperature (20-30 minutes) before opening the moisture barrier bag. If the slide was ordered with the Microarray Starter Kit it will already have a cassette mounted onto it. If not, affix a cassette to the slide.
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 60 rpm for 30 minutes. 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 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.
3. Immediately apply the glycan binding protein 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.
4. 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 (do not wrap the foil around the bottom as this could prevent the slide from lying flat). Incubate on the shaker for 1-3 hours at 60 rpm. If the samples can easily aggregate, shake at 100 rpm or higher 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 glycan-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.
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 60 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 secondary 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 30 minutes to 1 hour at 60 rpm. Shaking at faster speed such as 100 rpm 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 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 (do not wrap the foil around the bottom as this could prevent the slide from lying flat). Incubate on the shaker at 60 rpm for 30 minutes. Longer incubation time is acceptable, but not necessary.

Part 6 – Final wash and dry

1. Remove the sample 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.
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. Analyze data with microarray analysis software. If there is specific binding the signal intensity should be significantly higher than the negative control signal. Our standard method of comparing signal intensities is to quantify the median signal intensity data and subtract the background intensity.

Interpretation of Control Signals:

Negative Control (Print Buffer): The negative control 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.

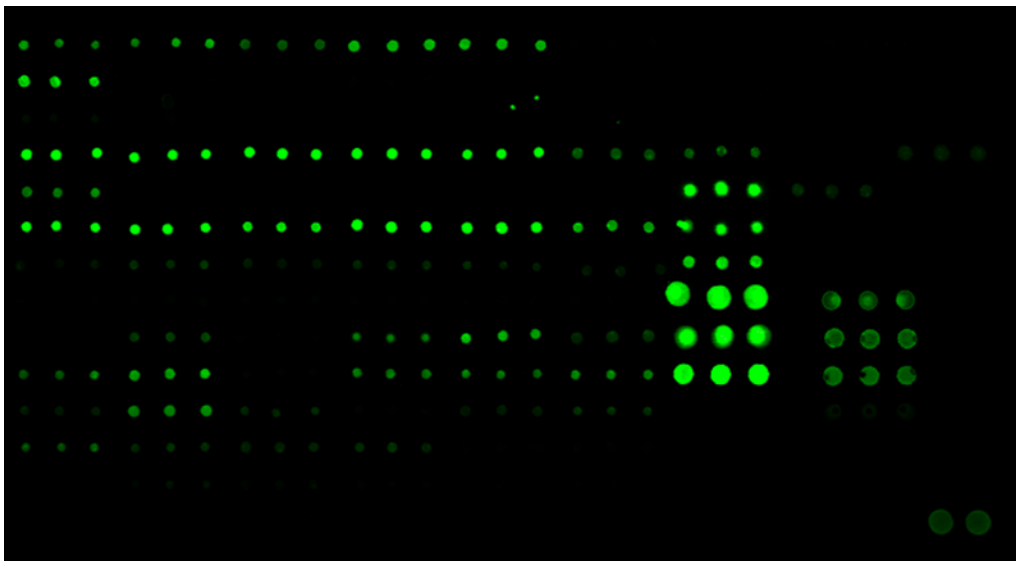
Marker: The array marker should show a strong fluorescence signal regardless of the assay. It is there primarily to aid with orientation of the array map during analysis.

Biotinylated Mannose (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.

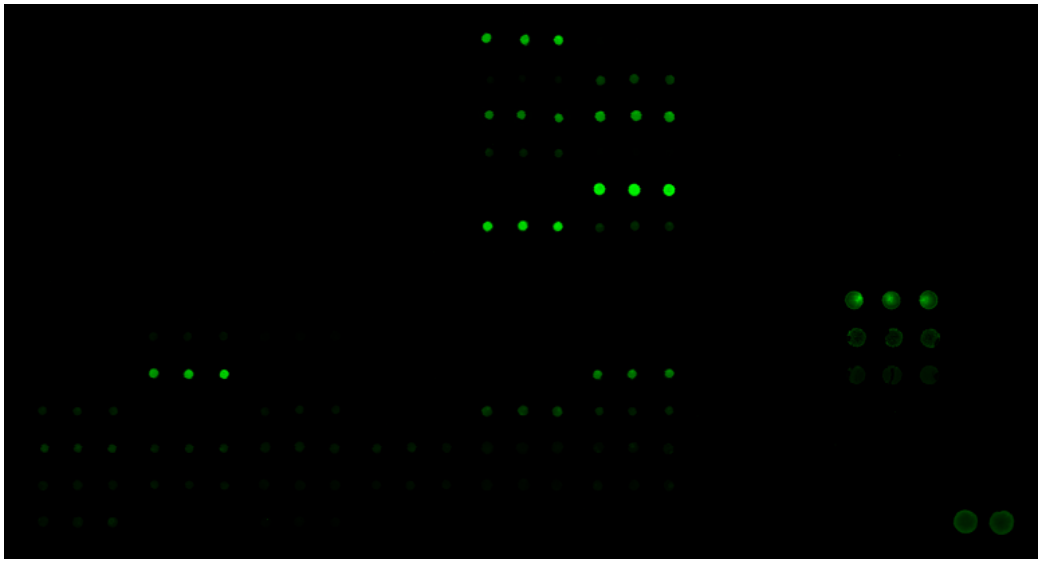
IgG (PC2, PC3, PC4): IgG is an antibody found in blood that is a primary component of humoral immunity. If the glycan-binding or secondary antibody sample is an anti-IgG antibody from human, mouse, or rabbit it should bind to the respective IgG control.

Typical Binding Assay Result from the 100 N-Glycan Array

Example 1: 100 N-glycan array on 8 subarray. A subarray assayed with glycan-binding protein biotinylated ConA lectin, followed by streptavidin-Cy3 conjugate. Array was scanned with a GenePix scanner at 475 PMT and 100% 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, as well as mannose-containing N-glycans.



Example 2: 100 N-glycan array on 8 subarray. A subarray assayed with glycan-binding protein biotinylated AAL lectin, followed by streptavidin-Cy3 conjugate. Array was scanned with a GenePix scanner at 475 PMT and 100% 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, as well as fucosylated N-glycans.



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