Bacterial Glycan Array User Manual

Z BIOTECH

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For Research Use Only

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Introduction

Lipopolysaccharides (LPS) are the highly immunogenic endotoxins on bacterial cells which are responsible for mediating the immune response to bacterial infection. Enriched on their cell membranes and secreted vesicles, lipopolysaccharides are the major determinants of infectious disease progression. The outer portion of the LPS - the O-antigen containing repeating glycan units - is the most variable portion of the LPS. This interactive epitope has various effects depending on the strain, often allowing the host to develop O-antigen specific protective immunity, or in some cases evolving to induce an immune response that actually helps protect the bacteria. In any case these highly involved glycan communicators are candidates for targeted antimicrobial agents or immune therapies. More research is needed to uncover the specific interactions these LPS have with the immune system in order to direct effective vaccine design.

The Bacterial Glycan Array is a research platform designed to efficiently test binding interactions with the O-antigens of bacterial LPS. Our version 1 microarray features 22 of these O-antigens purified from various bacterial strains. One microarray may be used to determine binding interactions for 8 to 16 samples each, whether the samples are sera, antibodies, or other glycan-binding 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.

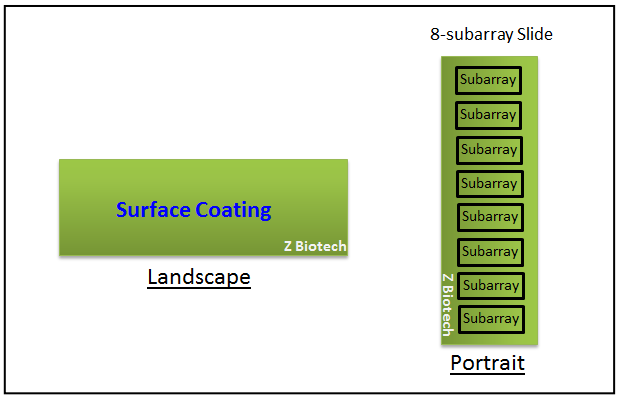
Handling and Storage

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

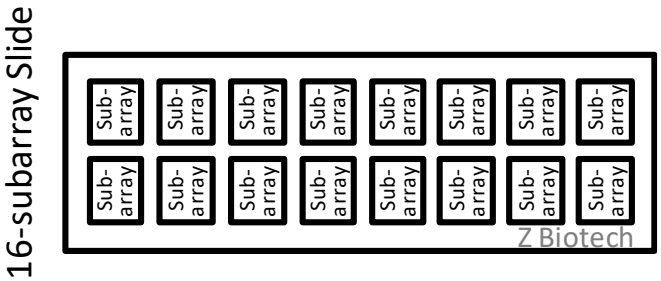
Array Map/Schematic

Bacterial Glycan Array slides have 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 on the following pages.

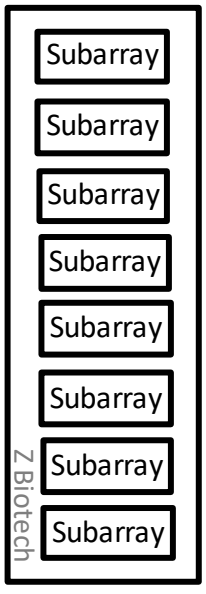


Array Map (16-subarray):

	1	2	3	4	5	6	7	8	9	10	11	12
1	EC1	EC1	EC1	EC1	EC1	EC1	EC2	EC2	EC2	EC2	EC2	EC2
2	EC3	EC3	EC3	EC3	EC3	EC3	EC4	EC4	EC4	EC4	EC4	EC4
3	EC5	EC5	EC5	EC5	EC5	EC5	EC6	EC6	EC6	EC6	EC6	EC6
4	EC7	EC7	EC7	EC7	EC7	EC7	EC8	EC8	EC8	EC8	EC8	EC8
5	EC9	EC9	EC9	EC9	EC9	EC9	SE1	SE1	SE1	SE1	SE1	SE1
6	SE2	SE2	SE2	SE2	SE2	SE2	SE3	SE3	SE3	SE3	SE3	SE3
7	SE4	SE4	SE4	SE4	SE4	SE4	SE5	SE5	SE5	SE5	SE5	SE5
8	SE6	SE6	SE6	SE6	SE6	SE6	ST1	ST1	ST1	ST1	ST1	ST1
9	KP1	KP1	KP1	KP1	KP1	KP1	SM1	SM1	SM1	SM1	SM1	SM1
10	PA1	PA1	PA1	PA1	PA1	PA1	CJ1	CJ1	CJ1	CJ1	CJ1	CJ1
11	PV1	PV1	PV1	PV1	PV1	PV1	PM1	PM1	PM1	PM1	PM1	PM1
12	NC1	NC1	NC1	NC1	NC1	NC1	NC2	NC2	NC2	NC2	NC2	NC2
13	PC1	PC1	PC1	PC1	PC1	PC1	PC2	PC2	PC2	PC2	PC2	PC2
14	PC3	PC3	PC3	PC3	PC3	PC3	PC4	PC4	PC4	PC4	PC4	PC4
15											Marker	Marker



Array Map (8-subarray):



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	EC1	EC1	EC1	EC1	EC1	EC1	EC2	EC2	EC2	EC2	EC2	EC2	EC3	EC3	EC3	EC3	EC3	EC3
2	EC4	EC4	EC4	EC4	EC4	EC4	EC5	EC5	EC5	EC5	EC5	EC5	EC6	EC6	EC6	EC6	EC6	EC6
3	EC7	EC7	EC7	EC7	EC7	EC7	EC8	EC8	EC8	EC8	EC8	EC8	EC9	EC9	EC9	EC9	EC9	EC9
4	SE1	SE1	SE1	SE1	SE1	SE1	SE2	SE2	SE2	SE2	SE2	SE2	SE3	SE3	SE3	SE3	SE3	SE3
5	SE4	SE4	SE4	SE4	SE4	SE4	SE5	SE5	SE5	SE5	SE5	SE5	SE6	SE6	SE6	SE6	SE6	SE6
6	ST1	ST1	ST1	ST1	ST1	ST1	KP1	KP1	KP1	KP1	KP1	KP1	SM1	SM1	SM1	SM1	SM1	SM1
7	PA1	PA1	PA1	PA1	PA1	PA1	CJ1	CJ1	CJ1	CJ1	CJ1	CJ1	PV1	PV1	PV1	PV1	PV1	PV1
8	PM1	PM1	PM1	PM1	PM1	PM1	NC1	NC1	NC1	NC1	NC1	NC1	NC2	NC2	NC2	NC2	NC2	NC2
9	PC1	PC1	PC1	PC1	PC1	PC1	PC2	PC2	PC2	PC2	PC2	PC2	PC3	PC3	PC3	PC3	PC3	PC3
10	PC4	PC4	PC4	PC4	PC4	PC4							Marker	Marker	Marker	Marker	Marker	Marker

Bacterial Glycan Identification List:

No.	ID	Item
1	EC1	*Escherichia coli* O111:B4
2	EC2	*Escherichia coli* O55:B5
3	EC3	*Escherichia coli* O26::B6
4	EC4	*Escherichia coli* J5 (Rc mutant)
5	EC5	*Escherichia coli* F583 (Rd mutant)
6	EC6	*Escherichia coli* O128:B12
7	EC7	*Escherichia coli* K-235
8	EC8	*Escherichia coli* EH100 (Ra mutant)
9	EC9	*Escherichia coli* O127:B8
10	SE1	*Salmonella enterica* serotype enteritidis
11	SE2	*Salmonella enterica* serotype minnesota
12	SE3	*Salmonella enterica* serotype typhimurium
13	SE4	*Salmonella enterica* serotype minnesota Re 595 (Re mutant)
14	SE5	*Salmonella enterica* serotype typhimurium SL1181
15	SE6	*Salmonella enterica* serotype abortus equi
16	ST1	*Salmonella typhosa*
17	KP1	*Klebsilella pneumoniae*
18	SM1	*Serratia Marcescens*
19	PA1	*Pseudomonas aeruginose* O10
20	CJ1	*Campylobacter jejuni* Penner O:19
21	PV1	*Proteus vulgaris* OX19
22	PM1	*Proteus mirabilis* OXK

Controls

NC1: Negative control for most glycans, print buffer

NC2: Negative control for PA1

PC1: Positive control 1, a biotinylated probe (0.01 mg/ml)

PC2: Postitive control 2, Human IgG (0.1 mg/ml)

PC3: Postitive control 3, Mouse IgG (0.1 mg/ml)

PC4: Postitive control 4, Rabbit IgG (0.1 mg/ml)

Array Marker: Anti-Human IgG, Cy3 (0.01 mg/ml) and anti-Human IgG, Alexa555 (0.01 mg/ml)

Materials Required

- Arrayed glass slide
- 16 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 (GAAB Item #10107)

Materials above are available for purchase

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

Preparation of assay samples:

Prepare glycan-binding samples or detection antibodies in a centrifuge tube by diluting with the GAAB buffer. For the fluorescent-labeled streptavidin we recommend a concentration of 1 μg/mL. For detection antibodies, we recommend a concentration of around 1-10 μg/ml. We generally recommend a range of 100 μg/ml to 0.1 μg/ml concentration for glycan-binding 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 testing different dilutions of samples to different wells of the array. In addition to testing a dilution range for your glycan-binding sample of interest, we recommend setting up control assays for any additional detection or secondary antibodies to ensure that any binding observed is specific to your sample of interest. Fluorescent signal due to specific binding to your sample of interest should be dose-dependent within the dynamic range of your sample dilution, and should have positive binding signal after 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. We recommend using 80-100 μL volume of sample per well for 16 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. 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, and there are no dry spots on the subarray after the sample is added.

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 to ensure full and even coverage of the printed area throughout incubation.
3. Cover the wells with adhesive film to prevent evaporation and incubate the slide on a 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 glycan-binding sample of interest is bacteria or cells, centrifuge samples to avoid adding irrelevant or aggregated 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. Briefly rinse each well with assay buffer and then remove it by pipette.
4. Immediately apply the glycan-binding sample of interest to each well. We recommend using 80 µL per well for 16 subarray cassettes 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. If the samples can easily aggregate, shake at higher speed to prevent 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 samples are fluorescent-labeled, 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, 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. 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 or fluorescent-labeled antibody (Sandwich Assay Format)

1. Unless this secondary antibody sample is bacteria or cells, centrifuge samples briefly to avoid adding irrelevant or aggregated particles to the array.
2. After completely removing the wash buffer immediately add the antibody to each well. We recommend using 80 µL per well for 16 subarray cassettes. Seal the wells with adhesive film and incubate on the shaker for 1 hour at 100 rpm. Shaking at faster speed may prevent aggregation. Longer incubation time is acceptable, but not necessary.

If the sample added is fluorescent-labeled, go to Part 6 – Final wash and dry. If the sample added is biotinylated, continue to Part 5 – Fluorescent staining.

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. 80 µL per well is recommended for 16 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. 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.
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 sample of interest should be both dose-dependent with your sample dilution (unless the sample 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 Controls (Print Buffer): The negative controls 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.

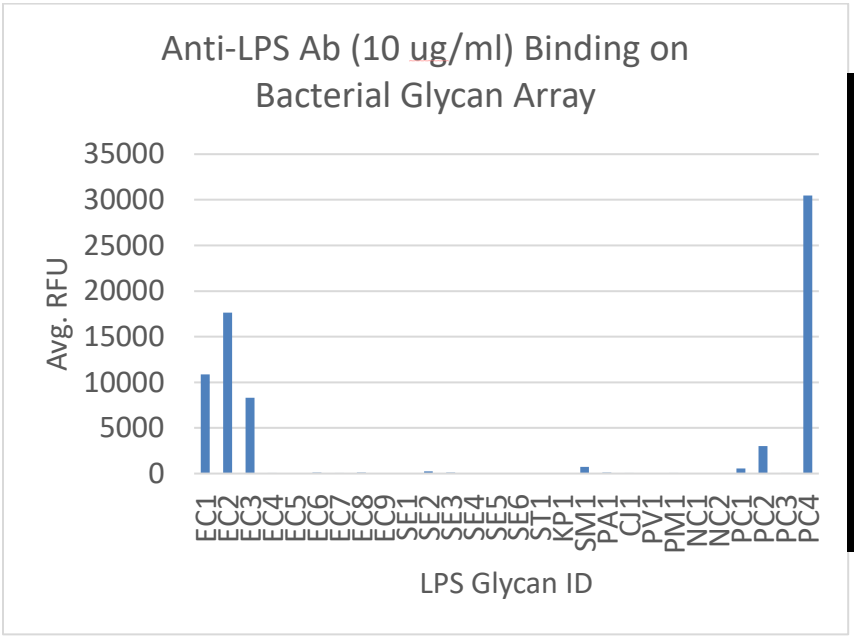
Positive Control (a biotinylated probe): This positive control will bind directly to the fluorescent labelled streptavidin. If your glycan-binding sample is already fluorescently labelled, or in any case where the addition of fluorescent labelled streptavidin to the array was not preformed (Part 5 –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

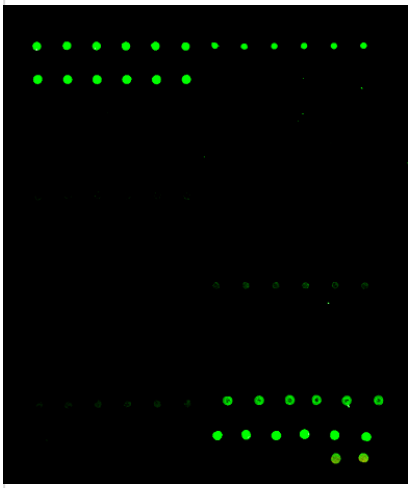
IgG (PC2, PC3, PC4): IgG is an antibody found in blood that is a primary component of humoral immunity. If the glycan-binding or secondary antibody sample is an anti-IgG from human, rabbit, or mouse it should bind to the respective IgG control.

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

Typical Binding Assay Result from the Bacterial Glycan Array

Example 1: Bacterial Glycan Array on 16 subarray format. A subarray assayed with anti-LPS antibody (10 µg/ml), followed by anti-rabbit IgG, Cy3 (5 µg/ml). The array was scanned with InnoScan 710 microarray scanner at 1 PMT and low laser power at 532nm wavelength. The positive control 4 (rabbit IgG) shows binding as expected.





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