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ab272785
Glycan Array 300

For identification of the specific glycan binding proteins in serum, plasma, cell culture supernatants, cell/tissue lysates or other body fluids.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Glycan Array 300 (ab272785) is the largest commercially available glycan array for screening protein-carbohydrate interactions.

This array will help researchers: 1) identify the glycans binding partners in biological samples, 2) identify whether target proteins are carbohydrate binding proteins, 3) probe binding of pathogens including bacteria, viruses and whole cells to glycans, 4) profile the substrate specificity of enzymes (glycosyltransferases, glycosidases, etc.); 5) profile the inflammatory immune response.

The 300 synthetic glycans featured in the new generation Glycan Glycan-300 array consists of four groups of targets including the 100 glycans from the Raybio Glycan-100 array and an additional 80 N-glycans, 50 glycolipid glycans, and 50 human milk oligosaccharide glycans. The included glycans have been frequently identified as structures showing important binding functions in the literature. For example, a variety of sialosides have been shown to bind to the influenza virus with a serotype-specific pattern. Galectins, which are involved in apoptosis, cell adhesion and T-cell activation suppression, function by binding beta-galactosides. Glycosphingolipids interact with growth factor receptors to modify signal transduction or with bacterial toxin subunits, such as Vero(Shiga) toxin, cholera toxin, and the heat labile E.coli toxins, to induce cell apoptosis. Additionally, oligosaccharides from human milk bind to microbes to promote growth of normal flora and inhibit pathogens in the gastrointestinal tract. This array provides a powerful tool for researchers in glycobiology and other fields.

Label-based Procedure

Incubation with biotin-labeled sample

Incubation with labeled streptavidin

Detection of signals

Data analysis

Sandwich-based Procedure

Incubation with sample

Incubation with biotinylated antibody

Incubation with labeled streptavidin

Detection of signals

Data analysis and graph

2. Materials Supplied and Storage

Store at -20°C in the dark immediately on receipt. After initial use, remaining reagents should be stored at +4°C to avoid repeated freeze-thaw cycles and may be stored for up to 3 months (Labeling Reagent, should be prepared fresh each time before use). Unused glass slides should be kept at -20 °C and repeated freeze-thaw cycles should be avoided (slides may be stored for up to 6 months).

Element	1-Slide kit	2-Slide kit	4-Slide kit	Storage temperature (before prep)	Storage temperature (after prep)
Dialysis Vials	8 vials	16 vials	32 vials	-20°C	RT
Labeling Reagent	1 vial	2 vials	4 vials	-20°C	+4°C
Stop Solution	50 µL	50 µL	2 x 50 µL	-20°C	+4°C
Glycan Array Glass Slide Assembly*	1 slide	2 slides	4 slides	-20°C	-20°C
Sample Diluent	8 mL	2 x 8 mL	4 x 8 mL	-20°C	+4°C
20X Wash Buffer I	30 mL	30 mL	2 x 30 mL	-20°C	+4°C
20X Wash Buffer II	30 mL	30 mL	2 x 30 mL	-20°C	+4°C
Cy5 equivalent dye-conjugated Streptavidin	2 vial	4 vials	8 vials	-20°C	+4°C
Slide Washer/Dryer	1	1	2	-20°C	RT
Adhesive device sealer	2	4	8	-20°C	RT
Labeling Buffer	30 mL	30 mL	2 x 30 mL	-20°C	+4°C
Floating Dialysis Rack	1	1	2	-20°C	RT

* Each slide contains 4 identical subarrays

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Detection antibodies of interest (For sandwich-based method only)

- Orbital shaker
- Laser scanner for fluorescence detection
- Aluminum foil
- 1.5 mL Polypropylene microcentrifuge tubes
- KCl, NaCl, KH_2PO_4 and Na_2HPO_4 (For label-based method only)
- Beaker, stir plate and stir bar

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Prepare fresh reagents immediately prior to use.

5.1 Cy5 Equivalent Dye-Streptavidin:

Briefly spin down the Cy5 equivalent dye-conjugated streptavidin tube immediately before use.
Add 1000 µL of Sample Diluent to Cy5 equivalent dye-conjugated streptavidin tube. Mix gently (do not store the stock solution for later use).

6. General Considerations

6.1 Label-Based vs. Sandwich-Based Method

Glycan Array 300 (ab272785) can be used with either a label-based method or with a sandwich-based method.

- In the **label-based** method, the proteins or antibodies in the sample are biotin labeled (via a simple reaction targeting primary amines), allowing direct detection on the array via a cy5 equivalent dye-conjugated biotin-streptavidin complex. A complete protocol and the primary materials for this procedure are included with the kit.
- The **sandwich-based** method is used for antibody-based detection of target proteins captured on the array. The user will need to supply the labeled reporter antibodies specific for their protein of interest. An example protocol for this procedure with a general "Antibody Cocktail" is included in this manual. Specific antibody concentrations and conditions will need to be determined by the end user.

6.2 Sample Preparation

We recommend the following parameters for your samples: 400 to 800 µL of 40X diluted serum, plasma, cell culture media, or other body fluid, or 50-500 µg/ml of protein for cell and tissue lysates.

Δ Note: If you experience high background or the readings exceed the detection range, further dilution of your sample is recommended.

6.3 Handling Glass Slides

6.3.1 The microarray slides are delicate. Please do not touch the surface of the slides with pipette tips, forceps or your fingers. Hold the slides by the edges only.

6.3.2 Handle the slides with latex free gloves in a clean environment.

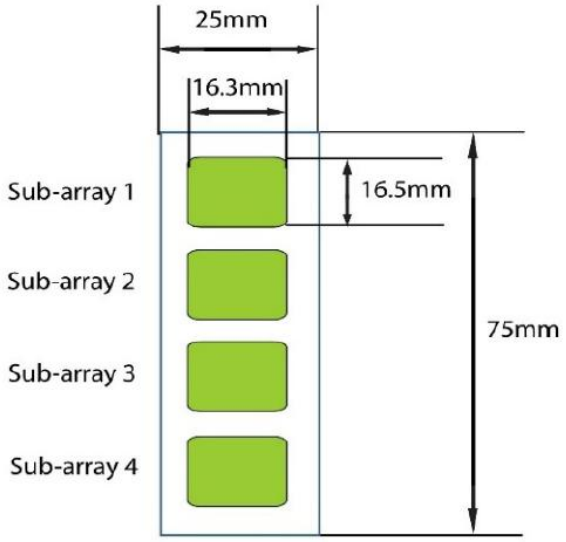
6.3.3 Do not remove the glass slide from the chamber assembly until Step 7.7.1 and take great care not to break the glass slide when doing so.

6.3.4 Permanent marker ink can significantly interfere with fluorescent signal detection. Never mark anywhere on the front (arrayed) side of the slide. It's best to avoid using marker completely, however if you need to number the slide, please add a small mark only on the back of the slide along the top or bottom edge using a green or blue ultra-fine point Sharpie® brand marker, only after the slide is completely dry.

6.3.5 Remove reagents/sample by gently applying suction with a pipette to corners of each chamber. Do not touch the printed area of the array, only the sides.

6.4 Layout of Glycan Array 300 Glass Slide

There are four identical sub-arrays on one slide.



6.5 Incubations and Washes

6.5.1 Cover the incubation chamber with adhesive film during incubation to prevent evaporation, particularly when incubation is more than 2 hours.

6.5.2 Avoid foaming during incubation steps and wash steps. Be sure to remove all bubbles from the sub-array surface.

6.5.3 Perform all incubation and wash steps with gentle rocking motion (~0.5 to 1 cycle/sec).

6.5.4 Avoid cross-contamination of samples to neighboring wells. To remove Wash Buffers and other reagents from chamber wells, you may invert the Glass Slide Assembly to decant, and aspirate the remaining liquid.

6.5.5 Several incubation steps such as step 7.4.3 (sample incubation), step 7.5.3 (detection antibody incubation), may be done overnight at 4°C. Please make sure to cover the incubation chamber tightly to prevent evaporation.

6.5.6 Unlike most Cy5 fluors, the Streptavidin-Conjugated Fluor used in this kit is very stable at room temperature (RT) and resistant to photobleaching on the hybridized glass slides. However, please protect glass slides from directly strong light and temperatures above RT.

7. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- **Δ Note:** Biotin Label-Based protocol starts here. For the Sandwich-Based protocol (using researcher's own detection antibody), start at Step 7.3 Dry the Glass Slide. Do not do Steps 7.1-7.2.

7.1 Dialysis of Sample

Δ Note: Samples must be dialyzed prior to biotin-labeling (Step 7.2).

7.1.1 Prepare enough dialysis buffer (1X PBS, pH=8.0) for all dialysis steps herein and after. To prepare 1 L dialysis buffer, dissolve 0.2 g KCl, 8 g NaCl, 0.2 g KH_2PO_4 and 1.15 g Na_2HPO_4 in 800 mL ddH_2O. Adjust pH=8.0 with 1M NaOH and adjust final volume to 1000 mL with ddH_2O.

7.1.2 Add each sample into a separate Dialysis Tube. Load 200 µL cell culture supernatant or 100 µL cell lysates or tissue lysate (1~2 mg/mL total protein) or 20 µL serum or plasma + 80 µL 1X PBS, pH=8 (5-fold dilution). Carefully place Dialysis Tubes into Floating Dialysis Rack.

Δ Note: If the samples appear to be cloudy, transfer the samples to a clean tube, centrifuge at 13,000 rpm for 20 minutes at 2-8°C. If the samples are still not clear, store them at -20°C for 20 minutes. Remove from the freezer, immediately centrifuge at 13,000 rpm for 20 minutes at 2-8°C.

7.1.3 Place Floating Dialysis Rack into at least 500 mL dialysis buffer in a large beaker. For more than 2 samples, make certain to use at least 300 mL dialysis buffer for each sample (more buffer will improve the efficiency of dialysis). Place beaker on a stir plate and dialyze for at least 3 hours at 4°C, stirring buffer gently. Then exchange the dialysis buffer and repeat dialysis for another 3 hours at 4°C. Transfer dialyzed sample to a clean Eppendorf tube. Spin dialyzed samples for 5 min at 10,000 rpm to remove any particulates or precipitants, and then transfer the supernatants to a clean tube.

Δ Note: The sample volume may change during dialysis.

Δ Note: Dialysis procedure may proceed overnight.

Δ Note: Determine the total protein concentration for cell culture supernatants or cell/tissue lysate after dialysis

procedure (Step 7.1.3). We recommended using a BCA total protein assay.

7.2 Biotin-labeling Sample

Δ Note: Amines (e.g., Tris, glycine) and azides quench the biotinylation reaction. Avoid contaminating samples with these chemicals prior to biotinylation.

7.2.1 Immediately before use, prepare 1X Labeling Reagent. Briefly spin down the Labeling Reagent tube. Add 100 µL 1X PBS into the tube, pipette up and down or vortex slightly to dissolve the lyophilized reagent.

7.2.2 Add 1X Labeling Reagent to dialyzed samples.

7.2.2.1 For labeling cell culture supernatants: transfer 180 µL dialyzed sample into a new tube. Add 36 µL of 1X Labeling Reagent Solution per 1 mg total protein in dialyzed cell culture supernatant. Mix well. For example, if sample's total protein concentration is 0.5 mg/mL you need to add 3.24 µL 1X Labeling Reagent to the tube of 180 µL dialyzed sample.
Δ Note: You need to biotin-label 360 µL of dialyzed sample if dilution of the biotin-labeled samples is 2-fold in Step 7.4.3.

7.2.2.2 For labeling serum or plasma: Add 22 µL of 1X Labeling Reagent Solution into a new tube containing 35 µL dialyzed serum or plasma sample and 155 µL Labeling Buffer.

7.2.2.3 For labeling cell or tissue lysates: transfer 30 µg (15 µL of 2 mg/mL) cell or tissue lysates into a tube and add Labeling Buffer for a total volume of 300 µL. Then add 3.3 µL of 1X Labeling Reagent Solution.

Δ Note: To normalize serum/plasma or cell/tissue lysate concentrations during biotinylation, measure sample volume before and after dialysis. Then adjust the volumes of dialyzed serum/plasma or cell/tissue lysates and Labeling Buffer to compensate. For example, if the sample volume doubles after dialysis, then use twice as much serum/plasma in the labeling reaction (70 µL) and reduce the Labeling Buffer to 120 µL.

7.2.3 Incubate the reaction solution at room temperature with gentle rocking or shaking for 30 minutes. Mix the reaction solution by gently tapping the tube every 5 minutes.

7.2.4 Add 3 µL Stop Solution into each reaction tube and immediately dialyze as directed in Steps 7.1.

Δ Note: Biotinylated samples can be stored at -20°C or -80°C until you are ready to proceed with the assay.

7.3 Dry the Glass Slide

Δ Note: Sandwich-Based protocol starts here.

7.3.1 Take out the package containing the Glycan Array Glass Slide Assembly and let the slide equilibrate to room temperature inside the sealed plastic bag for 20-30 minutes. Remove slide from the plastic bag; peel off the cover film, and let it air dry at room temperature for another 1-2 hours. Do not disassemble the Glass Slide from the chamber assembly.

Δ Note: Protect the slide from dust or other contaminants.

7.4 Blocking and Incubation

Δ Note: Glass slide should be completely dry before adding Sample Diluent to wells.

7.4.1 Block sub-arrays by adding 400µL Sample Diluent into each well and incubate at room temperature for 30 minutes. Ensure there are no bubbles on the array surface.

7.4.2 Immediately prior to sample incubation, spin biotin-labeled samples for 5 minutes at 10,000 rpm to remove any particulates or precipitates. Dilute samples with Sample Diluent.

Δ Note: Recommended dilution of the biotin-labeled samples with Sample Diluent prior to incubation is 2-10-fold for cell culture supernatants, 20-fold for serum/plasma or 30-fold cell/tissue lysate.

7.4.3 Decant buffer from each well. Add 400µL of sample to each well. Incubate arrays with gentle rocking or shaking at room temperature for 2-3 hours. (Longer incubation time is preferable if higher signal intensity is desired).

Δ Note: This step may be done overnight at 4°C for highest intensities.

7.4.4 Wash:

7.4.4.1 Based on number of samples and remaining protocol, calculate the amounts of 1x Wash Buffers I & II that are needed for each step of the protocol. Separately dilute required amounts of 20x Wash Buffer I and 20x Wash Buffer II with ddH_2O to 1x concentration. For example, if 12 mL of 1x

Wash Buffer I is needed then 600 µL of 20x Wash Buffer I would be diluted to a final volume of 12 mL.

7.4.4.2 Decant the samples from each well and wash each well 5 times (5 minutes each) with 800 µL of 1x Wash Buffer I at room temperature with gentle shaking. Completely remove wash buffer between each wash step.

7.4.4.3 (Optional for Cell and Tissue Lysates) Put the glass slide with frame into a box with 1x Wash Buffer I (cover the whole glass slide and frame with Wash Buffer I), and wash at room temperature with gentle shaking for 20 minutes.

7.4.4.4 Decant the 1x Wash Buffer I from each well, wash 2 times (5 minutes each) with 800 µL of 1x Wash Buffer II at room temperature with gentle shaking. Completely remove wash buffer between each wash step.

Δ Note: Incomplete removal of the wash buffer after each wash step may cause "dark spots". (i.e., background signal higher than that of the spot.)

7.5 Incubation with Biotinylated Detection Antibody (provided by researcher)

Δ Note: For the Label-Based protocol, go directly to Step7.6 Incubation with Cy5 Equivalent Dye-Streptavidin. Do not do Step 7.5.

7.5.1 If the researcher wishes to use their own antibody to detect specific bound proteins, we recommend using a biotinylated antibody at a dilution appropriate for Western blot. Optimal dilution must be determined by the researcher.

7.5.2 Dilute the detection antibody in Sample Diluent. Mix well and spin briefly.

7.5.3 Add 400 µL of the detection antibody to each well. Incubate at room temperature for 1-2 hours.

Δ Note: Longer incubation time is preferable for higher signals.

7.5.4 Decant the samples from each well and wash 5 times with 800 µL of 1x Wash Buffer I and then 2 times with 800 µL of 1x Wash Buffer II at room temperature with gentle shaking. Completely remove wash buffer between each wash step.

7.6 Incubation with Cy5 Equivalent Dye-Streptavidin

7.6.1 Prepare 1X Dye-conjugated Streptavidin:

7.6.1.1 Prepare Cy5 equivalent dye-conjugated streptavidin as described in section 5.1 immediately before use.

7.6.1.2 To prepare 1X Cy5-Conjugated Streptavidin add 200 µL of the concentrated Cy5-Conjugated Streptavidin stock solution into a tube with 800 µL of Sample Diluent. Mix gently.

7.6.2 Add 400 µL of 1X Cy5 equivalent dye-conjugated streptavidin to each well. Cover the incubation chamber with the plastic adhesive strips and cover the slide with aluminum foil to avoid exposure to light or incubate in dark room.

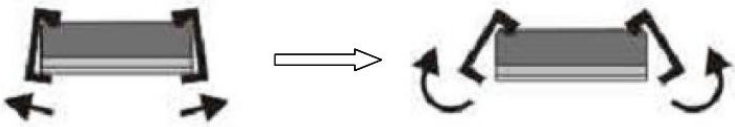
7.6.3 Incubate the slide with Cy5-Conjugated Streptavidin at room temperature for 1 hour with gentle rocking or shaking.

7.6.4 Decant the samples from each well and wash 5 times with 800 µL of 1x Wash Buffer I at room temperature with gentle shaking. Completely remove wash buffer in each wash step.

7.7 Fluorescence Detection

7.7.1 Disassemble the slide assembly by pushing clips outward from the slide side, as shown below. Carefully remove the slide from the gasket.

Δ Note: Be careful not to touch the surface of the array.



7.7.2 Gently place the slide in the slide Washer/Dryer (a 4-slide holder/centrifuge tube), add enough 1x Wash Buffer I (about 30 mL) to cover the whole slide, and then gently shake at room temperature for 15 minutes. Decant Wash Buffer I. Wash with 1x Wash Buffer II (about 30 mL) and gently shake at room temperature for 5 minutes.

7.7.3 Finally, wash the glass slide with 30 mL of de-ionized or distilled water for 5 minutes.

7.7.4 Remove water droplets completely by gently applying suction with a pipette. Do not touch the sub-array areas, only the sides of the slide.

Δ **Note:** Make sure the finished glass slide is completely dry before scanning or storage.

7.7.5 Imaging: The signals can be visualized through use of a laser scanner equipped with a Cy5 wavelength. Make sure that the signal from the spot containing the highest concentration receives the highest possible reading yet remains unsaturated.

Δ **Note:** Unlike most Cy5 fluors, the Streptavidin-Conjugated Fluorused in this kit is very stable at RT and resistant to photobleaching on completed glass slides. However, please protect glass slides from temperatures above RT and store them in the dark. Do not expose glass slide to strong light, such as sunlight or UV lamp.

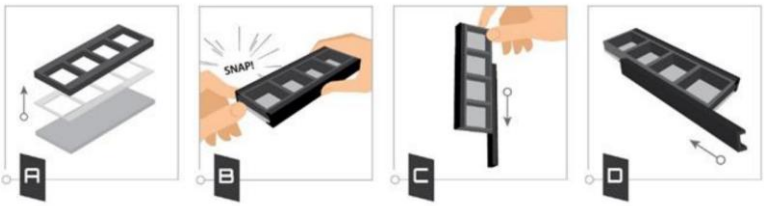
Δ **Note:** If you need to repeat any of the incubation after finishing the experiment, you must first re-assemble the glass slide into the incubation chamber by following step as shown in the figures below. To avoid breaking the printed glass slide, you may first want to practice assembling the device with a blank glass slide.

7.7.5.1 Apply slide to incubation chamber facing upward as in image A (below).

7.7.5.2 Gently snap one edge of a snap-on side as shown in image B.

7.7.5.3 Gently press other of side against lab bench and push in lengthwise direction (image C).

7.7.5.4 Repeat with the other side (image D).


A
SNAP!
B
C
D

8. Typical Data

8.1 Data Analysis

8.1.1 Data extraction can be done with most of the microarray analysis software.

8.2 Interpretation of Results:

Explanation of Controls Spots:

Positive Control spots (POS1 and POS2) are standardized amounts of biotinylated IgGs printed directly onto the array. All other variables being equal, the Positive Control intensities will be the same for each sub-array. This allows for normalization based upon the relative fluorescence signal responses to a known control, much as "housekeeping" genes or proteins are used to normalize results in PCR or Western blots, respectively.

Negative Control (NEG) spots contain a protein-containing buffer (used to dilute glycans printed on the array). Their signal intensities represent non-specific binding of Biotin-conjugated anti-Cytokines and/or the Cy5-Conjugated Streptavidin. Negative control signal intensities are usually very close to background signals in each sub-array.

The following figures show the Glycan Array 300 probed with biotin-labeled lectin mixtures and a serum sample. The images were captured using an Axon GenePix laser scanner. The strong signals in the upper left and lower right corners of each array are Positive Controls, which can be used to identify the orientation and help normalize the results between arrays. Interaction with different lectin mixtures, which have the ability to bind to different glycans with corresponding moieties such as aGal, aFuc, GlcNAc et al., the Glycan-300 array displays strong signals with different patterns. When incubated with a biotinylated serum sample, glycans in the array showed strong binding activity, indicating that the serum sample contains spectrometric proteins with binding activity to different glycans.

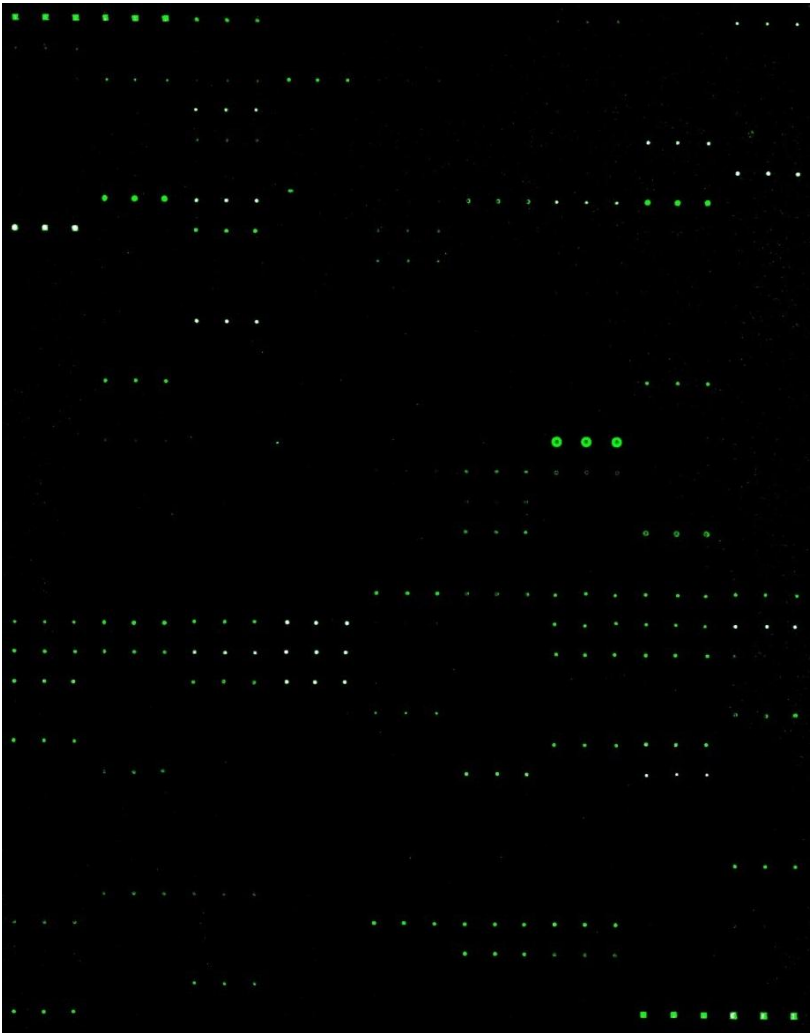


Figure 1: Biotinylated lectin mixture: BSL I, WGA, UEA I, and Con A (0.04 µg/mL)

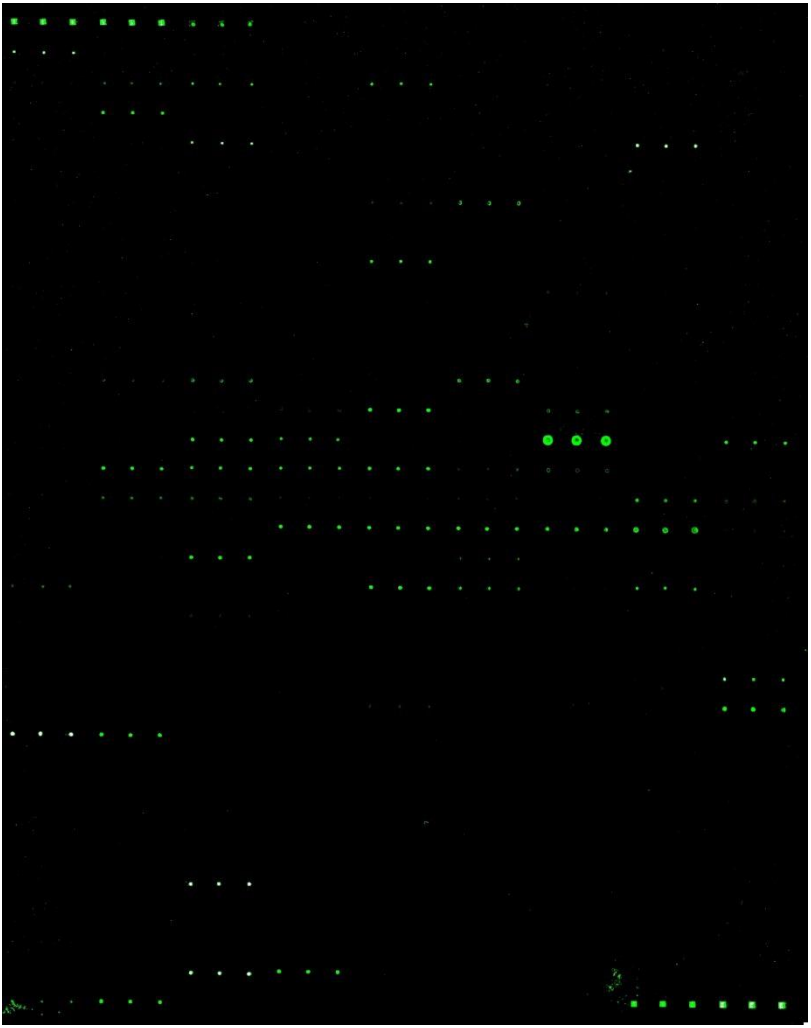


Figure 2: Biotinylated lectin mixture: SBA, PHA-E and PSA (0.4 µg/mL)

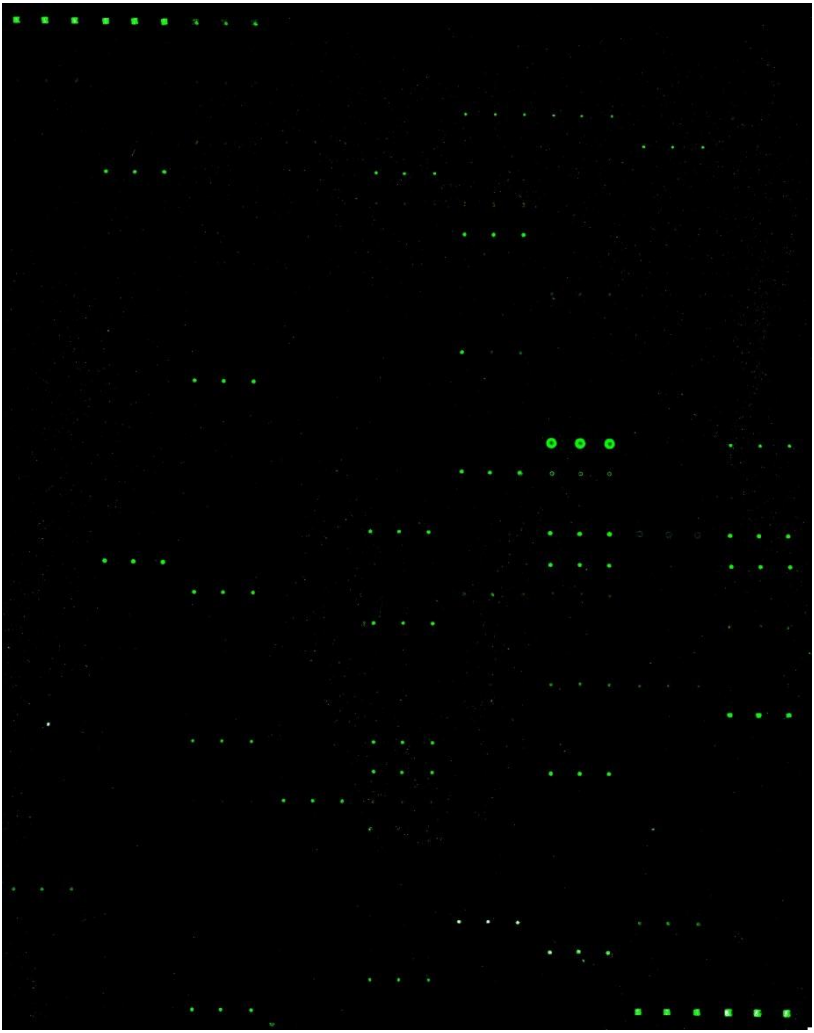


Figure 3: Biotinylated lectin mixture: DBA, PNA and RCA-I (0.4 µg/mL)

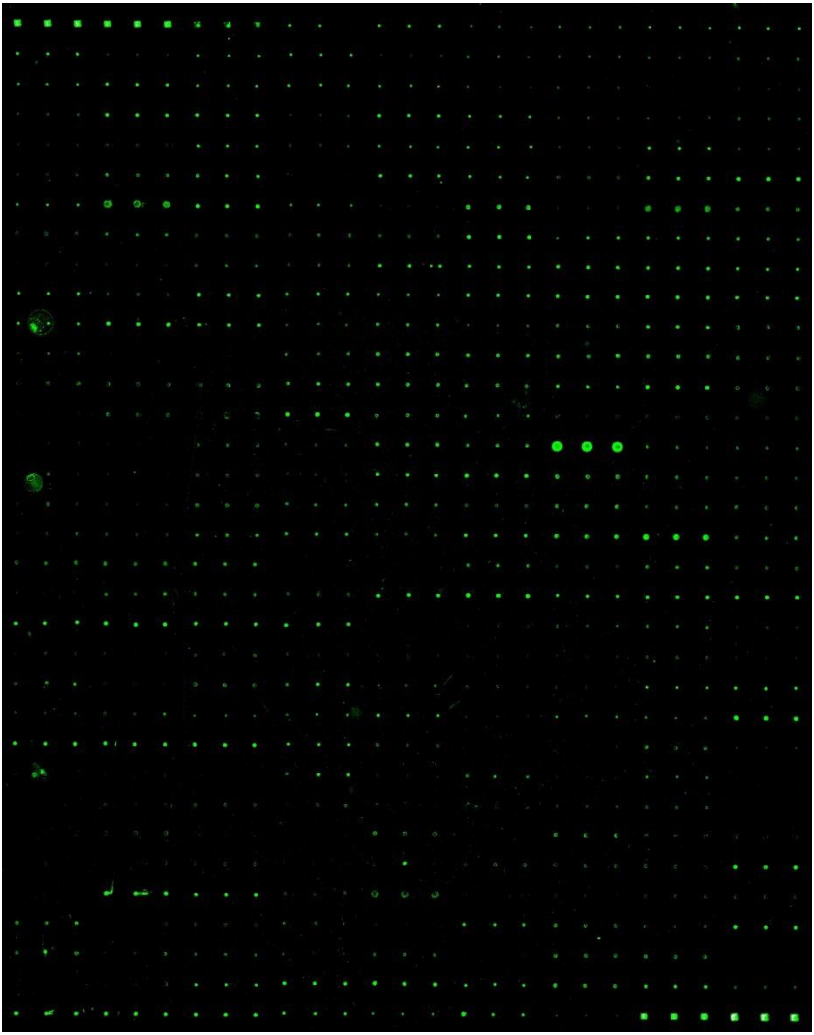


Figure 4: Biotinylated serum sample

Figure 5: Background control

If scanned using optimal settings, 2 distinct signal intensities will be seen: POS1>POS2. If these signals are of similar intensity, try increasing or decreasing laser power and/or signal gain settings.

8.3 Background Subtraction:

Once you have obtained fluorescence intensity data, you should subtract the background and normalize to the Positive Control signals before proceeding to analysis.

Most laser fluorescence scanner software has an option to automatically measure the local background around each spot. For best results, we recommend comparing signal intensities representing the MEDIAN background signals minus local background. If your resulting fluorescence signal intensity reports do not include these values (e.g., a column labeled as "MED532-B532"), you may need to subtract the background manually or change the default settings on your scanner's data report menu.

8.4 Normalization of Array Data:

To normalize signal intensity data, one sub-array is defined as "reference" to which the other arrays are normalized. This choice is arbitrary.
You can calculate the normalized values as follows:

$X(Ny) = X(y) * P1/P(y)$

Where:
P1 = mean signal intensity of POS spots on reference array
P(y) = mean signal intensity of POS spots on Array "y"
X(y) = mean signal intensity for spot "X" on Array "y"
X(Ny) = normalized signal intensity for spot "X" on Array "y"

Analysis software is available for use with data obtained using Glycan Array 300. With analysis software you can copy and paste your signal intensity data (with and without background) in, and it will automatically normalize signal intensities to the Positive Controls.

8.5 Threshold of significant difference in samples:

After subtracting background signals and normalization to Positive Controls, comparison of signal intensities between and among array images can be used to determine relative differences between samples or groups.
Any ≥1.5-fold increase or ≤0.65-fold decrease in signal intensity for a single analyte between samples or groups may be

considered a measurable and significant difference in expression, provided that both sets of signals are well above background (Mean background + 2 standard deviations, accuracy ≈ 95%).

9. Troubleshooting

Problem	Cause	Recommendation
Weak Signal	Inadequate detection	Increase laser power and PMT parameters
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Short incubation time	Ensure sufficient incubation time or change sample incubation step to overnight
	Too low glycan concentration in sample	Reduce amount of dilution or concentrate sample
	Improper storage of kit	Store kit as suggested temperature; Don't freeze/thaw the slide
Uneven Signal	Bubble formed during incubation	Handle and pipette solutions more gently; De-gas solutions prior to use
	Arrays are not completed covered by reagent	Prepare more reagent and completely cover arrays with solution
	Reagent evaporation	Cover the incubation chamber with adhesive film during incubation
General	Cross-contamination from neighboring wells	Avoid overflowing wash buffer
	Comet tail formation	Air dry the slide for at least 1 hour before usage
	Inadequate detection	Increase laser power that the highest concentration for each lectin receives the highest possible reading yet remains unsaturated
High Background	Overexposure	Lower the laser power
	Dark spots	Completely remove wash buffer in each wash step
	Insufficient wash	Increase wash time and use more wash buffer
	Dust	Minimize dust in work environment before starting experiment
	Slide is allowed to dry out	Take additional precautions to prevent slides from dying out during experiment

10. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)