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# ab272786
# Lectin Array 70

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Lectin Array 70 Kit datasheet:
www.abcam.com/ab272786
(use www.abcam.cn/ab272786 for China, or www.abcam.co.jp/ab272786 for Japan)

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

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

# Table of Contents

# 1. Overview

Lectin Array 70 (ab272786) uses standard glass slides each spotted with 14 wells of identical lectin arrays.

This array will help researchers: 1) identify and profile the glycans in their samples; 2) determine whether their biomarker of interest has glycan moieties, and; 3) find specific glycan binding ligands in biological samples.

Lectins are glycan-binding proteins which have been purified from trees, beans and some fruits. They are highly specific for a given glycan based on their sequence and the different sugar unit structures the glycan contains. For Lectin Array 70 (ab272786) one standard glass slide is spotted with 14 identical lectin arrays, 1 in each well. Each lectin, together with the positive controls is arrayed in duplicate. The slide comes with a 16-well removable gasket which allows for the processing of 14 samples using one slide. Four slides can be nested into a tray, which matches a standard microplate and allows for automated robotic high-throughput processing of 56 arrays simultaneously. This array provides a powerful new tool for glycosylation determination, drug discovery and biomarker development; all with limited sample volumes required.

**Detection Method:** Fluorescence with laser scanner: Cy3 equivalent dye

**Sample Volume:** 50 – 100 µL per array

**Reproducibility:** CV <20%

**Assay duration:** 6 hours

## 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).

| Component | 1-Slide kit | 2-Slide kit | 4-Slide kit | Storage temperature (before prep) | Storage temperature (after prep) |
|---|---|---|---|---|---|
| Dialysis Vials | 28 vials | 56 vials | 112 vials | -20°C | +4°C |
| Labeling Reagent | 4 vials | 8 vials | 16 vials | -20°C | +4°C |
| Labeling Buffer | 1 x 8 mL | 2 x 8 mL | 4 x 8 mL | -20°C | +4°C |
| Stop Solution | 1 x 50 µL | 2 x 50 µL | 4 x 50 µL | -20°C | +4°C |
| Lectin Array Glass Slide Assembly | 1 slide | 2 slides | 4 slides | -20°C | -20°C |
| Sample Diluent | 1 x 8 mL | 1 x 8 mL | 2 x 8 mL | -20°C | +4°C |
| 20X Wash Buffer I | 1 x 30 mL | 1 x 30 mL | 2 x 30 mL | -20°C | +4°C |
| 20X Wash Buffer II | 1 x 30 mL | 1 x 30 mL | 2 x 30 mL | -20°C | +4°C |
| Cy3 equivalent dye-conjugated Streptavidin | 1 vial | 2 vials | 4 vials | -20°C | -20°C |
| Slide Washer/Dryer | 1 | 1 | 2 | -20°C | +4°C |
| Adhesive device sealer | 2 | 4 | 8 | -20°C | +4°C |
| Floating Dialysis Rack | 2 | 4 | 8 | -20°C | +4°C |

## 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.

# 6. General Considerations

## 6.1 Label-Based vs. Sandwich-Based Method

Lectin Array 70 (ab272786) can be used with either a label-based method or with a sandwich-based method.

- The **label-based** method is used to biotinylate samples containing proteoglycans and glycoproteins for direct detection on the array via a Cy3 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 detection of glycocalyx elements (glycolipids, glycoproteins, etc.) captured on the array. The user will need to supply the labeled reporter antibodies specific for the glycocalyx elements 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

Use serum-free conditioned media if possible.
If serum-containing conditioned media is required, it is highly recommended that complete medium be used as a control since many types of sera contain glycocalyx elements.
We recommend the following parameters for your samples:
50 to 200 µL of original or diluted serum, plasma, cell culture media, or other body fluid, or 50-100 µL cell or tissue lysates with 1~2 mg/mL total protein concentration.

**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 Do not touch the surface of the slides, as the microarray slides are very sensitive. Hold the slides by the edges only.

6.3.2 Handle all buffers and slides with latex-free gloves.

6.3.3 Handle the glass slide in a clean environment.

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.

## 6.4 Incubation

6.4.1 Completely cover array area with sample or buffer during incubation.

6.4.2 Avoid foaming during incubation steps.

6.4.3 Perform all incubation and wash steps under gentle rotation.

6.4.4 Cover the incubation chamber with the adhesive film during incubation to prevent evaporation, particularly when incubation is more than 2 hours or <70 µL of sample or reagent is used.

6.4.5 Several incubation steps such as step 7.4.1 (blocking), step 7.4.3 (sample incubation), detection antibody incubation, or step 7.5.3 (Cy3 equivalent dye-streptavidin incubation) may be done overnight at 4°C. Please make sure to cover the incubation chamber tightly to prevent evaporation.

# 7. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- **Δ Note:** For the Sandwich-Based protocol, 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 minutes 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.

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.

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 bag containing the glass slide from the box, and let the slide equilibrate to room temperature inside the sealed plastic bag for 20-30 minutes. Then, remove slide from the plastic bag; peel off the cover film, and let it air dry at room temperature for another 1-2 hours.

**Δ Note:** Incomplete drying of slides before use may cause the formation of "comet tails".

## 7.4 Blocking and Incubation

7.4.1 Add 100 µL Sample Diluent into each well and incubate at room temperature for 30 minutes to block slides.

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, 20-100 fold for serum/plasma or 30-100 fold cell/tissue lysate, however, optimization is recommended for the best results.

7.4.3 Decant buffer from each well. Add 100 µL of sample to each well. Incubate arrays at room temperature for 1-2 hours. (Longer incubation time is preferable if higher signal intensity is desired).

**Δ Note:** We recommend using 50 to 100 µL of original or diluted serum, plasma, conditioned media, or other body fluid, or 50-500 µg/mL of protein for cell and tissue lysates. Cover the incubation chamber with adhesive film during incubation if less than 70 µL of sample or reagent is used.

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

**Δ Note:** For the Sandwich-based protocol, it's recommended to do optimization to determine appropriate dilution of non-biotinylated samples for incubation.

7.4.4 Wash:

7.4.4.1 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 150 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 150 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 Cy3 Equivalent Dye-Streptavidin

**Δ Note:** For the Sandwich-based protocol, follow below steps after secondary antibody incubation if biotinylated secondary antibody is used. If fluorescence conjugated secondary antibody is used, skip steps 7.5.1-7.5.3 and continue from step 7.5.4 after incubation of secondary antibody. Appropriate dilution of secondary antibody should be determined before incubation.

7.5.1 Briefly spin down the Cy3 equivalent dye-conjugated streptavidin tube.

7.5.2 Add 1.4 mL of Sample Diluent to Cy3 equivalent dye-conjugated streptavidin tube. Mix gently.

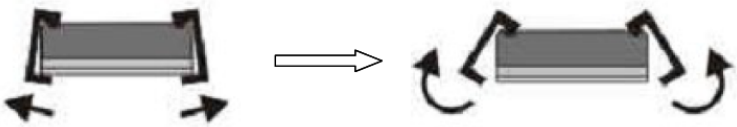
7.5.3 Add 80 µL of Cy3 equivalent dye-conjugated streptavidin to each well. Cover the slide with aluminum foil to avoid exposure to light or incubate in dark room. Incubate at room temperature for 1 hour.

7.5.4 Decant the samples from each well, and wash 5 times with 150 µL of 1x Wash Buffer I at room temperature with gentle shaking. Completely remove wash buffer in each wash step.

## 7.6 Fluorescence Detection

7.6.1 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.



7.6.2 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.

7.6.3 Remove liquid droplets completely by one of the following ways:

7.6.3.5 Put the glass slide into the Slide Washer/Dryer, and dry the glass slide by centrifuging at 1,000 rpm for 3 minutes without cap.

7.6.3.6 Or, dry the glass slide by a compressed $N_2$ stream.

7.6.3.7 Or gently apply suction with a pipette to remove water droplets. Do not touch the sub-array areas, only the sides of the slide.

7.6.4 Imaging: The signals can be visualized through use of a laser scanner equipped with a Cy3 wavelength. Make sure that the signal from the spot containing the highest concentration receives the highest possible reading, yet remains unsaturated.

**Δ Note:** If the signal intensity for different lectins vary greatly in the same array, we recommend using multiple scans, with a higher PMT for low signal lectins, and a low PMT for high signal lectins.

# 8. Typical Data

## 8.1 Data Analysis:

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

## 8.2 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"

## 8.3 Detection of Glycans on a Purified Protein:

In this application, the Lectin Array 70 (ab272786) was used to detect specific glycosylations of purified Horseradish Peroxidase (HRP). Lectins BANLEC, BC2L-A, CALSEPA, GNA, HHA, NPA, PA-IIL, and PALa showed strong signals after incubation with 3.3 µg/mL Biotin-HRP followed by detection with streptavidin-fluorescence-dye (Figures 1, 2 and 3). The fluorescence signals from BANLEC, BC2L-A, CALSEPA, GNA, HHA, NPA, PA-IIL, and PALa were blocked in a concentration-dependent manner by HRP itself (Figures 1 and 3), indicating that the signals were generated by lectin-HRP binding. These eight lectins are known to exhibit specific binding to mannose, which indicates that HRP contains mannose. After adding increasing amounts of mannose, the signal from BANLEC, BC2L-A, CALSEPA, GNA, HHA, NPA, PA-IIL, and PALa were reduced (Figures 1 and 2). The reduction in signals from increasing concentrations of mannose confirms that HRP protein contains mannose in its glycocalyx. Additionally, the two lectins AAL and RS-FUC (fucose binding specificity) also showed strong interaction with HRP, which indicates the fucosylation of HRP. Overall, the results of the Lectin Array 70

were consistent with published literature regarding HRP glycosylation.

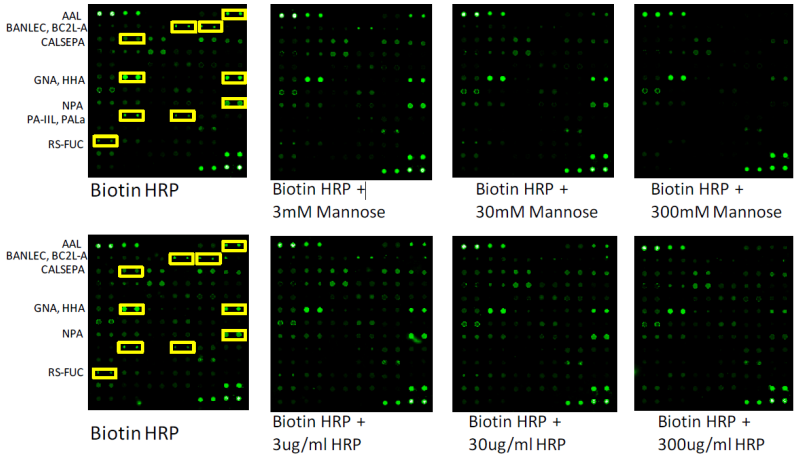



Figure 1:

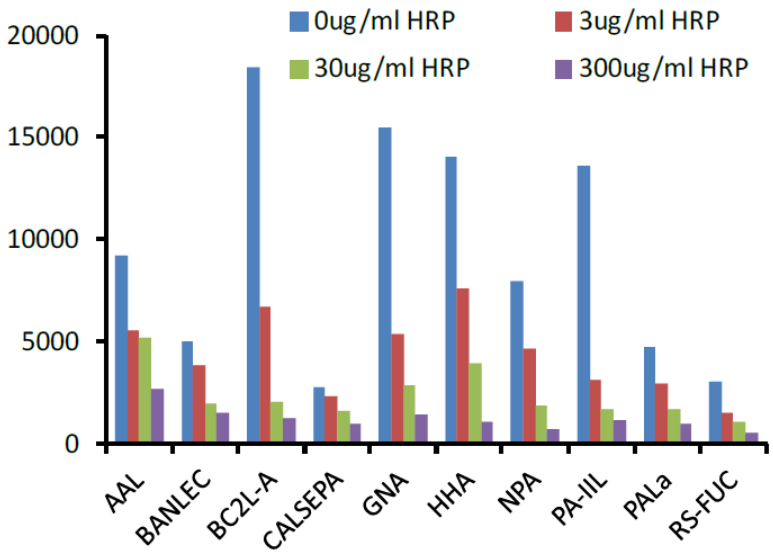



Figure 2:

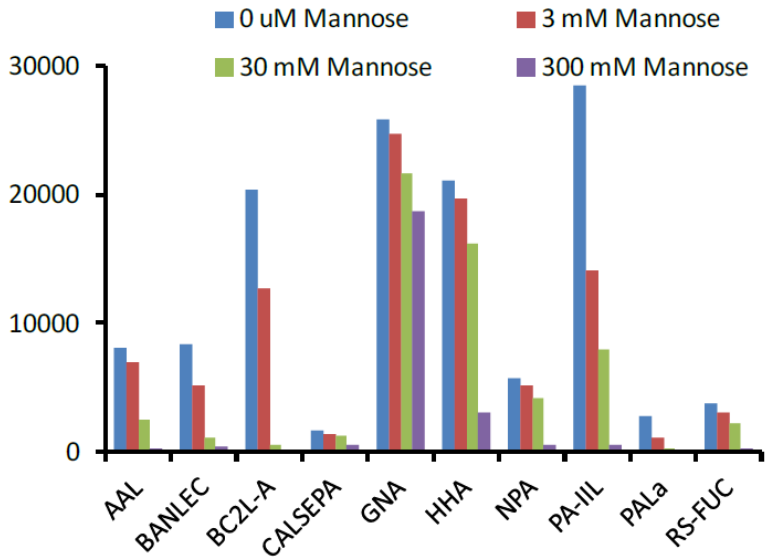



Figure 3:

## 8.4 Profiling of a Serum Sample:

Using the lectin array, we can discover the different glycoprotein profiles of the serum samples, cell lysates, or purified glycoprotein. The images below show the profiles of the glycans from different types of samples including human serum, recombinant glycoproteins human HE4, AFP, mouse TTF, purified human IgG, and bacterial cell lysates OF DH-5a, DE3 detected by Biotin labeling and Fluorescence dye-streptavidin.

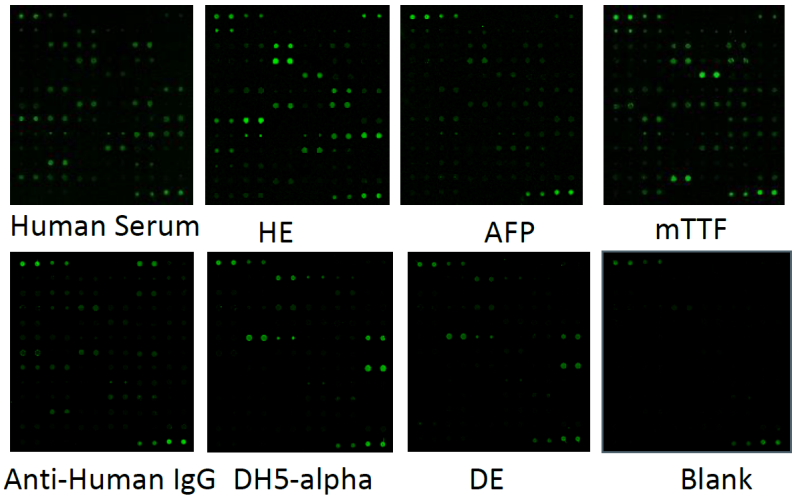



Figure 4:

## 8.5 Other Applications:

Quantitative analysis of lectin-glycoprotein interactions, Example: a concentration series of glycoproteins detected with the lectin array could reveal concentration dependent effects of lectin-glycan binding;
Determine the profile of bacterial cell-surface glycans; Cell lysate from bacteria can be biotinylated and hybridized to the lectin array. Analysis of the binding pattern and correlation with the known carbohydrate-binding specificities of the lectins can determine the glycans on the cell membrane.

# 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)