

Antibody Microarray

User's Guide



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INTRODUCTION

Antibody Microarray is a high-throughput ELISA based platform for efficient protein expression profiling, screening, and comparison between normal, diseased, or treated samples. Full Moon BioSystems' antibody arrays allow researchers to detect and analyze hundreds of native proteins simultaneously on a single slide, saving precious resources and reducing the number of variables that affect experimental outcome. Our unique collection of antibody arrays includes phospho-specific arrays for studying phosphorylation events, comprehensive exploratory arrays for examining hundreds of proteins in a single experiment, and pathway arrays for studying highly relevant proteins in specific research fields. Suitable samples include cell lysates, fresh/froze/FFPE tissue lysates, serum, culture supernatant and bodily fluids.

ANTIBODY ARRAYS

The antibodies on the array are covalently immobilized on a high-quality glass surface coated with our proprietary 3-D polymer materials, which are designed to promote high binding efficiency and specificity. All arrays are printed on standard-size microscope slides, and each slide contains one complete array. The arrays utilize fluorescent detection and can be scanned on all microarray scanners that are compatible with 76 x 25 x 1 mm (3 inch x 1 inch x 1 mm) slides.

Each set of antibody arrays contains two identical array slides – one slide can be used for a control sample, and the other for a treated sample.

To maximize data reliability, each antibody on the array is printed with replicates. Pathway arrays (<400 antibodies) contain six replicates. Larger arrays, including Explorer Antibody Array, Phospho Explorer Array, and Signaling Explorer Array, contain two replicates for each antibody.

To see a list of the antibodies featured in a specific array and their reactivity information, visit our website at www.fullmoonbio.com and select the array of your choice. Go to the Documentation section to view Antibody List and Array Layout.

Multiple positive markers and negative controls are included in each array. Positive markers contain Cy3 labeled antibodies to mark the boundaries of the array. Positive controls include beta-actin and GAPDH. Negative controls contain BSA. Empty spots contain no material, and their signal intensity may be used as background signal for data analysis.

ANTIBODY ARRAY ASSAY KIT

The Antibody Array Assay Kit is designed for easy and reliable processing of Full Moon BioSystems' antibody arrays. It provides the major reagents required to perform protein extraction, biotinylation, conjugation and detection. The reagents are convenient, easy to use, and optimized to work with our antibody arrays.

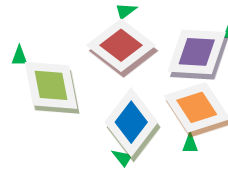
Each 2-reaction kit provides sufficient reagents to process two array slides. Each 20-reaction kit provides the reagents to process 20 slides.

HOW IT WORKS

Protein Extraction from cells,
tissues, or bodily fluids



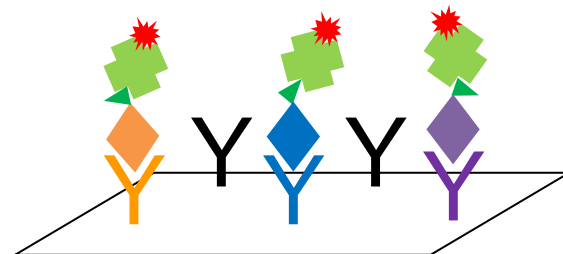
Biotinylation of Proteins



Protein Conjugation to
Antibody Array



Detection by
Dye-Streptavidin



The ELISA based antibody array platform involves four major steps: 1) Protein extraction with non-denaturing extraction buffer; 2) Biotinylation of protein samples; 3) Incubate labeled samples with antibody array; and 4) Detection by dye conjugated streptavidin.

Proteins used in the assay are not denatured, and native tertiary structures and multiprotein complexes are intact. Due to inaccessible target epitopes on the protein, this may lead to certain proteins undetected and increase the chance for false negatives.

EXPERIMENTAL CONSIDERATIONS

- All reagents and materials are intended for research use only.
- Handle the slides by holding the area with barcode labels. Do not touch the slide surface.
- Use extra care; Any variation in buffers, operator, pipetting technique, washing technique, and incubation time or temperature can alter the performance of the kit.
- Only use reagents and materials recommended by this user's guide. Do not substitute buffers or solutions from other sources.
- Do not allow arrays dry out between blocking, coupling, and washing. It can cause high background.
- Wash the arrays extensively with Wash Solution and water to remove excess residual reagents from the slide surface.
- The reagents provided in the Antibody Array Assay Kit do not contain protease inhibitors. To prevent protein degradation, you should work quickly and proceed diligently towards the array analysis step once you start the extraction. Alternatively, you may use inhibitors if you prefer or plan to store the proteins for a week or longer.
- Always wear gloves while handling any reagents.

COMPONENTS

Antibody Arrays

Material/Reagent	Quantity	Purpose	Storage Condition
Antibody Microarray	2 slides	Microarray	4 °C (6 months)

Antibody Array Assay Kit (sold separately from the arrays)

Catalog No.	Description	Quantity
KAS02	Antibody Array Assay Kit, 2 Reactions	2 Reactions
KAS20	Antibody Array Assay Kit, 20 Reactions	20 Reactions

Material/Reagent	Quantity		Purpose	Storage Condition
	2-Rxn Kit	20-Rxn Kit		
Biotin Reagent	1 mg	5 x 1mg	Labeling	-20 °C
Blocking Reagent	60 mL	600 mL	Blocking	4 °C
Coupling Chamber	1	5	Coupling	RT
Coupling Reagent	12 mL	120 mL	Coupling	-20 °C
Detection Buffer	60 mL	600 mL	Detection	4 °C
DMF	200 uL	1 mL	Labeling	4 °C
Dry Milk	1.8g & 0.36g	18g & 3.6g	Coupling	4 °C
Extraction Buffer	1.5 mL	15 mL	Cell and tissue lysis	4 °C
Labeling Buffer	2 mL	20 mL	Labeling	4 °C
Lysis Beads	2 tubes	20 tubes	Cell and tissue lysis	RT or 4 °C
Spin Columns	2 sets	20 sets	Buffer Exchange	RT
Stop Reagent	100 uL	1 mL	Stop labeling reaction	4 °C
10X Wash Buffer	100 mL	500 mL x 2	Washing	4 °C

ADDITIONAL MATERIALS REQUIRED

- 1X PBS, pH=7.4
- Dye conjugated streptavidin
 - Cy3-Streptavidin is commonly used. It can be purchased from any vendors including GE Healthcare: PA43001; Invitrogen: S-32355 (Alexa Fluor 555 streptavidin); Sigma-Aldrich: S6402, and others.
 - Other dye labeled streptavidin can be used in place of Cy3-streptavidin provided that the dye is compatible with your array scanner.
- 50 mL conical tube with cap
- Centrifuge with 1.5 mL microtube rotor
- Milli-Q Grade Water or dd H₂O
- Orbital shaker
- Petri dishes, 100 x 15mm (9 cm in diameter)
- Slide drying (only one option is required)
 - By compressed air: compressed nitrogen/clean air supplied by a central line or from a cylinder. Do not use canned air duster or heat gun/blower.
 - By centrifugation: centrifuge with 50 mL swinging bucket rotor
- UV Spectrophotometer
- Vortexer
- Microarray scanner compatible with 3 x 1 inch (76 x 25 mm) slides -- Optional
 - If you don't have access to a scanner, you can send the finished array slides to our lab for scanning. Visit www.fullmoonbio.com/services/array-scanning-image-analysis/ for details.

REAGENT PREPARATION

REAGENTS TO WARM BEFORE USE

Blocking Reagent Coupling Reagent Wash Buffer	Warm to 25-30°C in a water bath.
Biotin Detection Buffer DMF Dry Milk Labeling Buffer Stop Reagent	Warm to room temperature.

2-RXN Kit (KAS02)

1	1X Wash Solution	Make 1:10 dilution. In a one-liter reagent bottle, add 100 mL of 10X Wash Buffer to 900 mL of dd H ₂ O. Shake to mix.
2	Blocking Solution	Add 1.8 g of Dry Milk to 60 mL of Blocking Reagent. Shake to mix. Be sure the milk powder is completely dissolved. Use within one week.
3	Coupling Solution	Add 0.36 g of Dry Milk to 12 mL of Coupling Reagent. Shake to mix. Be sure the milk powder is completely dissolved. Use within one week.

20-RXN Kit (KAS20)

1	1X Wash Solution	Make 1:10 dilution. For example, add 100 mL of 10X Wash Buffer to 900 mL of ddH ₂ O to make 1L of 1X Wash Solution. Shake to mix.
2	Blocking Solution	If you plan to perform 20 assays within one week, add 18 g of Dry Milk to 600 mL of Blocking Reagent. Be sure the milk powder is completely dissolved. For two assays, aliquot 60 mL of Blocking Reagent and add 1.8 g of Dry Milk. Shake to mix. Use within one week.
3	Coupling Solution	If you plan to perform 20 assays within one week, add 3.6 g of Dry Milk to 120 mL of Coupling Reagent. Be sure the milk powder is completely dissolved. For two assays, aliquot 12 mL of Coupling Reagent and add 0.36 g of Dry Milk. Shake to mix. Use within one week.

ASSAY PROTOCOL – DETECTION BY CY3-STREPTAVIDIN

IMPORTANT – PREPARE AND WARM REAGENTS BEFORE USE**A. Sample Preparation**

Note: It is recommended cell and tissue lysates be prepared with the mild, non-denaturing Extraction Buffer provided in the Antibody Array Assay Kit (KAS02).

Other protein extraction and lysis procedures and buffers may be used. Because high concentrations of certain compounds adversely affect biotinylation of protein samples, extraction buffers with Tris and detergents should be limited to no more than 50mM Tris, 0.1% SDS, 1% Triton X-100, or 1% NP-40. Please contact us if you are unsure about your lysis buffer.

Note: The reagents provided in the Antibody Array Assay Kit do not contain protease or phosphatase inhibitors. Commonly used protease and/or phosphatase inhibitors (such as Roche's inhibitor cocktail) may be added to Extraction Buffer to prevent protein degradation or dephosphorylation.

I. Cells

1. **Adherent Cells:** Remove media and wash the culture with ice cold 1X PBS 3 – 5 times. Remove remaining PBS and add 100 – 200 uL of Extraction Buffer to 1 to 5 million cells. Detach cells using a scraper and transfer the cells and remaining supernatant to a clean microcentrifuge tube. Add one tube of Lysis Beads.

Suspension Cells: Transfer media containing cells to a clean tube. Pellet the cells by centrifugation at 500 x g for 2 minutes at 4°C. Remove media completely without disrupting the cells. Wash the pellet with ice cold 1X PBS followed by centrifugation. Repeat 3 times to ensure complete removal of media. Discard supernatant. Add Extraction Buffer and one tube of Lysis Beads to the cells. The amount of Extraction Buffer needed should be determined by the number of cells harvested. For 1 – 5 million cells, use 100 - 200 uL of Extraction Buffer.

Important: Only use PBS to wash the cells. Avoid using trypsin or other reagents.

Note: When working with primary cells or low protein yield cells, reduce extraction buffer amount, or increase cell quantity accordingly.

2. Vortex rigorously for 30 seconds to 1 minute. Incubate on ice for 10 minutes. Repeat 5 times.
3. Centrifuge the mixture at 10,000 x g for 5 minutes at 4°C.
4. Transfer all liquid to a clean tube. Discard the beads.

5. Centrifuge the new tube with liquid at 18,000 x g for 15 – 20 minutes at 4°C.
6. The supernatant on top should look colorless and transparent as water. Transfer the clear supernatant to a clean tube.

Important: If the supernatant appears to be cloudy, centrifuge again at 18,000 x g for 15 to 20 minutes at 4°C. Check again. If the supernatant is still not clear, store the lysate at -70°C for 10 to 20 minutes. Immediately centrifuge at 18,000 x g for 15 to 20 minutes at 4°C after removal from freezer. Save the top clear layer and discard the rest. The supernatant should look colorless and transparent as water.

7. Proceed immediately to Step B (Buffer Exchange/Lysate Purification).

II. Tissues

1. Wash tissues with ice cold 1X PBS with vortexing. Remove and discard PBS. Repeat 3 – 5 times.

Important: If blood in the tissues gets in the lysate, it will lead to high background on the arrays. Be sure to remove blood from the tissues completely. Increase the number of PBS washes if necessary. When blood has been completely removed, the tissues should appear white, and the PBS wash solution should appear clear and colorless.

2. Add one tube of Lysis Beads to 10 – 40 mg of tissues.
3. Add Extraction Buffer to the tissues. The amount of Extraction Buffer needed should be determined by the amount of tissue harvested. For 10 – 20 mg of tissue, use 100 uL of Extraction Buffer; for 20 – 40 mg of tissue, use 200 uL of Extraction Buffer.
4. Vortex rigorously for 30 seconds to 1 minute. Incubate on ice for 10 minutes. Repeat 5 times.
5. Centrifuge the mixture at 10,000 x g for 5 minutes at 4°C.
6. Transfer all liquid to a clean tube. Discard the beads.
7. Centrifuge the new tube with liquid at 18,000 x g for 15-20 minutes at 4°C.
8. The supernatant on top should look colorless and transparent as water. Transfer the clear supernatant to a clean tube.

Important: If the supernatant appears to be cloudy, centrifuge again at 18,000 x g for 15 to 20 minutes at 4°C. Check again. If the supernatant is still not clear, store the lysate at -70°C for 10 to 20 minutes. Immediately centrifuge at 18,000 x g for 15 to 20 minutes at 4°C after removal from freezer. Save the top clear layer and discard the rest. The supernatant should look colorless and transparent as water.

9. Proceed immediately to Step B (Buffer Exchange/Lysate Purification).

III. Serum/Plasma

1. Centrifuge the serum/plasma sample at 18,000 x g for 10 – 15 minutes at 4°C.

2. Transfer 3 – 4 uL of the clear, pale-yellow liquid to a new tube and proceed directly to Step D (Protein Labeling).

Important: After centrifugation, a thin milky film often collects at the top of serum/plasma sample. It is important that the milky film is not transferred to the new tube. Helpful tips: Do not disturb the milky film. While pressing and holding down the pipette plunger, insert the pipette tip directly through the milky film, then release the plunger to aspirate the clear sample below. Upon removing the pipette tip from liquid, wipe off the tip with a Kimwipe before releasing the sample in a new tube.

B. Buffer Exchange/Lysate Purification

1. Quickly spin the column on a mini-centrifuge to ensure dry gel has settled to the bottom of the column.
2. Remove the screw cap and reconstitute the column by adding 500 uL of Labeling Buffer.
3. Replace the cap and vortex vigorously for about 5 seconds. Remove air bubbles by sharply tapping the bottom of the column. Allow at least 2 – 3 hours of room temperature hydration time before using the column.

Note: If the column was stored at 4°C, allow the column to reach to room temperature before use.

4. After hydration, loosen the screw cap and then break off column's bottom stopper.
5. Place the column into a wash tube. Spin the column in its wash tube at 1500 x g for 1 minute.
6. Discard excess fluid in the wash tube. Return the column to its wash tube.
7. Add 300 uL of Labeling Buffer to the column. Spin at 1500 x g for 1 minute.
8. Discard the excess fluid and wash tube. Place the column into a collection tube. (Do not allow the gel material to dry excessively. Process the sample within the next few minutes.)

Note: The sample volume capacity of each spin column is 100 uL.

9. Transfer up to 100uL of protein extract by carefully dispensing the sample directly onto the center of the gel bed at the top of the column without disturbing the gel surface. Do not touch the sides of the columns with the reaction mixture or the pipette tip since this can reduce purification efficiency.
10. Spin the column in its collection tube at the 1500 x g for 2 minutes.
11. Purified protein will collect at the bottom of the collection tube. Discard the spin column.
12. Proceed immediately to the next step.

C. Lysate quantification and QC

1. Lysate quantification: Measure protein concentration of the lysate sample by UV absorbance

spectroscopy (A280), BCA assay, Bradford assay, or other quantification methods.

UV Absorbance A280: Measure lysate sample's absorbance (OD). Use Labeling Buffer or water as blank.

Note: The desired absorbance is 3 OD or higher. If the OD for your sample is too low, the sample must be concentrated at 4°C in a vacuum centrifuge, such as SpeedVac, or using YM-10 filters (Millipore Corporation).

Note: Only the absorbance reading (OD) is needed. This reading will be used in Step D to determine the amount of sample used for labeling and coupling. Conversion to protein concentration (mg/ml) is not necessary. Most A280 assays assume 1 OD = 1 mg/mL. This conversion is not accurate for lysate samples because non-protein components (such as, nucleic acids and insoluble matters) may interfere with the assay.

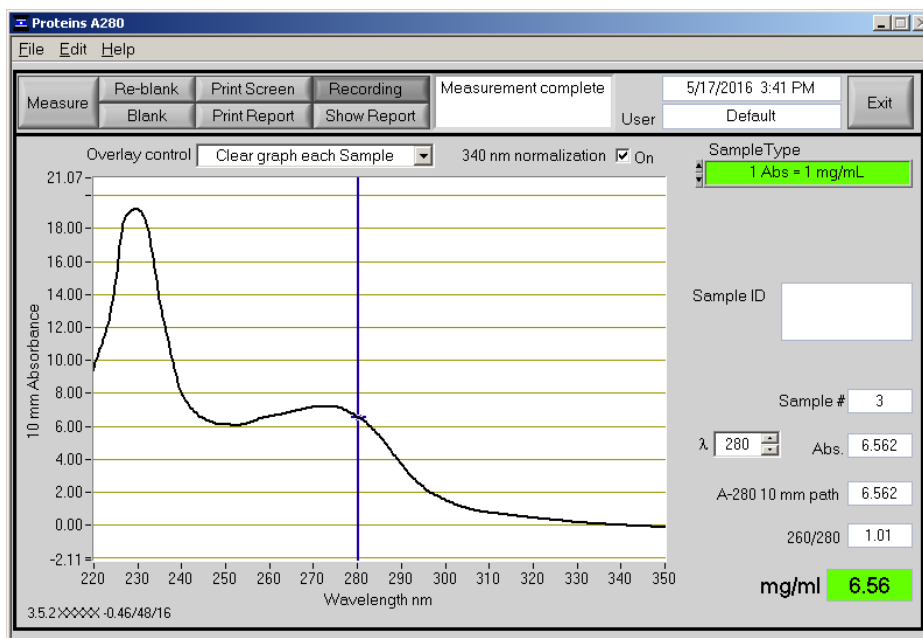
BCA and Bradford assays: either assay can be used to determine lysate sample's protein concentration. The minimum protein concentration required is 2 mg/ml.

2. Lysate Quality Control by A280 Assay – Optional, but highly recommended. This step can be performed at the same time when UV absorbance A280 is measured in Step 1 above.

The quality of lysate sample directly affects assay results. The lysate sample should be as clear and transparent as water. Cloudy or unclear lysate sample will result in low labeling efficiency and high background. A280 spectrum from a UV spectrometer (e.g., Nanodrop) is a good way to determine whether the lysate sample has sufficient clarity.

When UV A280 is measured, a clear lysate sample produces two well separated peaks at 200-230nm and 240-280nm. If the peaks are not well separated, it indicates the lysate is not sufficiently clear. To improve lysate quality, store the lysate at -70°C for 10 to 20 minutes.

Remove from the freezer and immediately centrifuge at 18,000 x g for 15 to 20 minutes at 4°C. Save the top clear layer and discard the rest.



This is an UV absorbance spectrum of a clear cell lysate. One peak is observed at 230nm, and a second peak is observed at 270nm. The two peaks are clearly separated. This indicates that the lysate is clear and ready for the next step.

3. Proceed immediately to the next step (Protein Labeling) or store the lysate at -80°C.

D. Protein Labeling – Biotinylation of Protein Samples

1. Biotin Preparation
 - a. Briefly centrifuge Biotin Reagent before use.
 - b. Add 100 μ L of DMF (*N,N*-Dimethylformamide) to 1 mg of Biotin Reagent. (The biotin concentration will be 10 μ g/ μ L.) Mix by vortexing, then quickly spin it down. Label this solution as Biotin/DMF.
2. Labeling
 - a. Determine the amount and volume of sample needed for the protein/antibody coupling reaction (Step F).

Lysates – For most lysate samples, 50 – 150 OD of protein may be used for each reaction. (If BCA or Bradford assay was used to measure protein concentration, use 30 – 100 μ g of protein.)

 - Use the protein concentration obtained from Step C to determine the volume of lysate sample needed.
 - Example: Assume 80 OD of lysate sample is planned for the protein/antibody coupling

reaction. If the lysate concentration is 4 OD, then the volume of lysate needed will be 20 uL (80 OD/4 OD = 20 uL).

- Aliquot and transfer the amount of lysate sample needed to a new tube. (This aliquot should be no more than 50 uL.)

Serum/Plasma – Continue with the sample prepared in Step A.III.

- b. Add Labeling Buffer to the sample to bring the total volume to 75 uL.
- c. Add 3 uL of the Biotin/DMF solution to the sample. Incubate the mixture at room temperature for 1 – 2 hours. Vortex the mixture every 10 minutes.

Note: Remaining Biotin/DMF solution may be stored at -20°C for future use.

- d. Add 35 uL of Stop Reagent. Mix by vortexing and quickly spin down. Incubate for 30 minutes at room temperature with mixing.
- e. Proceed immediately to the next step, or store the biotinylated sample at -80°C for future use.

E. Blocking

Pre-blocking preparation: Remove antibody arrays from refrigeration. Do not open the pouch. Allow the arrays (with packaging) to warm up to room temperature for 60 minutes. Then remove the pouch and allow the slides to dry for 30 minutes or longer. Depending on the ambient temperature and humidity, adjust warm-up and drying time accordingly.

High humidity locations (relative humidity greater than 50%): Remove antibody arrays from refrigeration. Do not open the pouch. Place the arrays (with packaging) in an oven at 30°C for 60 minutes. Remove the pouch, continue to allow the slides to dry completely in the oven for additional 10 minutes.

Important: Before starting Step 1, ensure Blocking Solution (See “Reagent Preparation”) has been warmed to room temperature and dry milk is completely dissolved.

1. Add 30 mL of Blocking Solution to a 100 x 15 mm Petri dish.
2. Submerge one slide in Blocking Solution. The side with a barcode label faces up.
3. Incubate on an orbital shaker rotating at 55 rpm for 30 to 45 minutes at room temperature.
4. Rinse the slide **extensively** with Milli-Q grade or DI water as follows:
 - a. Place the slide in a 50-ml conical tube. Fill the tube with 45 mL of water. Close the cap. Shake for 10 seconds. Discard the water.
 - b. Repeat 10 times.

Important: It is critical to rinse the slide extensively to completely remove Blocking Solution from the slide surface. A clean slide will ensure a uniform and low background. After the last rinse cycle, inspect the thin layer of water left on the slide surface. It should look uniformly smooth across the entire surface. If it appears spotty, repeat Step 4.

5. Shake off excessive water on the slide surface. Proceed immediately to the next step.

Note: Do not allow the slide to dry out. If you are not ready to start the coupling reaction, place the slide in a conical tube filled with clean water.

F. Coupling

Note: Protein Coupling Mix (Step F.1) may be prepared in advance so that you can start coupling immediately after blocking.

Important: Before starting Step 1, ensure Coupling Solution (See "Reagent Preparation") is at room temperature and dry milk is completely dissolved.

1. In a new tube, combine 6 mL of Coupling Solution and biotinylated sample from Step D. Vortex briefly to mix. Label it as "Protein Coupling Mix."
2. Place the slide in Well 1 (or any clean well) of the Coupling Chamber.
3. Slowly pour all 6 mL of Protein Coupling Mix over the slide. The slide should be completely submerged. Cover the Coupling Chamber with its lid.
4. Incubate on an orbital shaker rotating at 35 rpm for 1 – 2 hours at room temperature.
5. Transfer the slide to a 100x15 mm Petri dish containing 30 mL of 1X Wash Solution (See "Reagent Preparation"). Set the shaker speed to 55 rpm, continue for 10 minutes at room temperature. Discard the wash solution once shaking is completed.
6. Add 30 mL of fresh 1X Wash Solution to the same Petri dish containing the slide. Set the shaker speed to 55 rpm, continue for 10 minutes at room temperature. Discard the wash solution. Repeat this step one more time.
7. Rinse the slide **extensively** with Milli-Q grade or DI water as follows:
 - a. Place the slide in a 50-mL conical tube. Fill the tube with 45 mL of water. Close the cap. Shake for 10 seconds. Discard the water.
 - b. Repeat 10 times.

Important: It is critical to rinse the slide extensively to completely remove Coupling Solution from the slide surface. After the last rinse cycle, the thin layer of water left on the slide surface should look uniformly smooth across the entire surface. If it appears spotty, repeat Step 7.

8. Shake off excessive water on the slide surface and proceed to the next step immediately.

Note: Do not allow the slide to dry out. If you are not ready for the next step, place the slide in a conical tube filled with clean water.

G. Detection

1. Add 60 μ L of Cy3-streptavidin (0.5 mg/mL) to 60 mL of Detection Buffer.

Note: If the Cy3-streptavidin is in dry form, add biological grade ultrapure water to make 0.5

mg/mL.

2. Pour 30 mL of Cy3-streptavidin detection solution into a 100x15 mm Petri dish.
3. Submerge the slide in the Cy3-streptavidin detection solution. Incubate on an orbital shaker rotating at 35 rpm for 20 minutes at room temperature in the dark or covered with aluminum foil.
4. Transfer the slide to a new 100x15 mm Petri dish containing 30 mL of 1X Wash Solution. Set the shaker speed to 55 rpm, continue for 10 minutes at room temperature. Discard the wash solution once shaking is completed.
5. Add 30 mL of fresh 1X Wash Solution to the same Petri dish containing the slide. Set the shaker speed to 55 rpm, continue for 10 minutes at room temperature. Discard the wash solution. Repeat this step one more time.
6. Rinse the slide **extensively** with Milli-Q grade or DI water as follows: Place the slide in a 50-mL conical tube. Fill the tube with 45 mL of water. Close the cap. Shake for 10 seconds. Discard the water. Repeat 10 times.

Important: It is critical to rinse the slide extensively to completely remove Detection Solution from the slide surface. After the last rinse cycle, the thin layer of water left on the slide surface should look uniformly smooth across the entire surface. If it appears spotty, repeat Step 6.

7. Hold the slide with your fingers, shake off excess water from the slide.
8. Dry the slide with compressed nitrogen (or air) or by centrifugation.

Note: The goal is to remove water from the slide as quickly as possible.

By compressed air: Do not use compressed air in a can (for example, desktop air duster). Compressed air or nitrogen from a cylinder tank or an outlet on the fume hood is adequate. The air pressure should be less than 40 psi. Point the air nozzle at a 30° angle, one inch away from the slide surface. Starting from one end of the slide, push the water off of the surface. Repeat for the back side of the slide.

By centrifugation: Place the slide in a 50-mL conical tube. Centrifuge the tube, uncapped, at 1000 x g for 5 minutes.

9. The slide is now ready for scanning.

Note: If you do not have access to a microarray scanner, you can send the slides to our lab for scanning, free of charge. Visit www.fullmoonbio.com/services/array-scanning-image-analysis/ for details.

To prepare the slides for shipping, place the slides back in the slide holder. Cover the slide holder with aluminum foil to protect the slides from light. Send the package at room temperature. Please include the array information and your contact information (name, organization, phone and email address) in the package.

Shipping address:

Attn: Array Scanning Service
Full Moon BioSystems, Inc.
754 North Pastoria Avenue
Sunnyvale, CA 94085
United States

Phone: 408-737-1702

Email: support@fullmoonbio.com

ARRAY SCANNING

Microarray scanners that are compatible with 76mm x 25mm (3in x 1in) microscope slides may be used to scan Full Moon BioSystems' antibody arrays.

Recommended Scanning Resolution: 10um or higher (10um, 5um, etc.)

Commonly Used Compatible Array Imaging Systems

Manufacturer	Product Name	Required Accessory
Agilent Technologies	DNA Microarray Scanner SureScan Microarray Scanner	
Alpha Innotech	AlphaScan™ Microarray Scanner NovaRay™ Detection Platform	
Applied Precision	arrayWoRx®e Biochip Reader	
Aurora Photonics	PortArray 5000™	
Azure Biosystems	Sapphire and Sapphire Biomolecular Imager	
Biomedical Photometrics	The DNAscope™ HR	
Bio-Rad	VersArray ChipReader™	
GE Healthcare, formerly Amersham Biosciences	Typhoon™ 8610 and newer	Microarray Slide Holder Kit
Genewave	AmpliReader™ 4600 Microarray Reader	
InDevr	Vidia™ Microarray Imaging System	
INNOPSYS	InnoScan® Microarray Scanner	
Miltenyi Imaging GmbH	SensoSpot® Fluorescence Microarray Analyzer	4-slides Holder Adapter
Molecular Devices, formerly Axon Instruments	GenePix® Microarray Scanner All models	
PerkinsElmer, formerly Packard Bioscience	ProScanArray® HT Microarray Scanner ProScanArray® Microarray Scanner ScanArray® GX Microarray Scanner ScanArray® GX PLUS Microarray Scanner	
Tecan	LS Reloaded™ Versatile Scanner PowerScanner™ Microarray scanner	
Vidar Systems	Revolution™ 4200 Microarray Scanner	

Array Scanning Service

Our Antibody Array Scanning Service is free of charge for customers who purchase our antibody arrays but have no access to a microarray scanner. With this service, you will receive original raw image files in tiff format by email. The images may be analyzed using suitable image quantification software.

To use this service, the antibody array assay must be completed using Cy3 or Cy5-streptavidin or equivalent. Please refer to the wavelength table below when choosing alternative dyes.

	Cy3	Cy5
Excitation wavelength	~550 nm	~650 nm
Emission wavelength	~570 nm	~670 nm

This service may be ordered independently or with [Image Analysis Service](#).

IMAGE ANALYSIS

Antibody array images can be analyzed with image quantification software, including analysis software provided by scanner manufacturers (e.g., GenePix Pro, Agilent Feature Extraction), third-party software, or open-source programs (e.g., [ImageJ](#)).

Consumer grade picture viewing software programs (e.g., Microsoft Picture Manager) are not suitable for viewing or analyzing microarray images.

GenePix Array List (GAL) File

GAL file describes the size and position of blocks, the layout of feature-indicators in them, and the names and identifiers of the printed substances associated with each feature-indicator. When a GAL file is loaded, grids are generated automatically for spot quantification.

GAL file for each array is provided to aid image analysis. They can be found under the [Support](#) section on www.fullmoonbio.com. Parameters in the GAL files are provided for images scanned at 10 μ m resolution in portrait orientation. For images scanned at different resolutions, modify the parameters accordingly. For arrays scanned in other orientations, images must be rotated in order to use the GAL files. (A rotation guide for images acquired on Agilent microarray scanners can be found under the [Support](#) section on our website.)

For image analysis software programs that are incompatible with GAL files, grid files must be generated manually using array parameters and array map. Array parameters can be found in the [User's Guide](#) section on <https://www.fullmoonbio.com/support/userguide/>. Array Map for each array can be found

under the “Documentation” tab of each array’s webpage. Please refer to your analysis software’s user guide for instructions on how to generate a grid file.

Image Analysis Service

Our Array Image Analysis Service is available to users who have acquired array images through our Array Scanning Service but are unable to analyze antibody array images. Analysis results are delivered by email in 2-7 business days. For more details, visit our website at <https://www.fullmoonbio.com/services/array-image-analysis/>.

For questions related to antibody arrays, please contact our technical support team:

Phone: 408.737.2875

Email: support@fullmoonbio.com