

Antibody Array Assay Service Guide

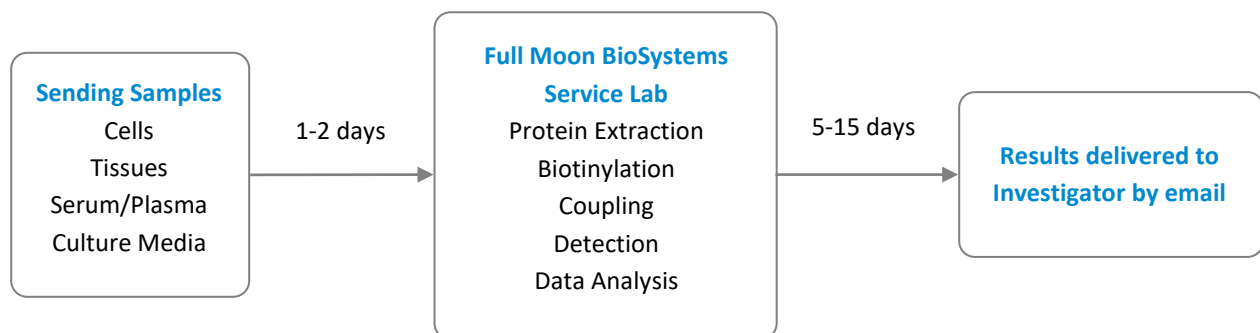


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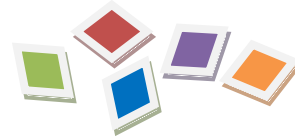
INTRODUCTION

Antibody microarray is an ELISA based high-throughput platform for sensitive, efficient, and accurate protein expression profiling, screening, and comparison between normal, diseased, or treated samples. Our Antibody Array Assay Service allows researchers to send samples to our dedicated laboratory in Sunnyvale, California for complete analysis by a team of experienced scientists. Researchers can choose from a wide range of [antibody microarrays](#) to study protein changes and phosphorylation events.



HOW ANTIBODY ARRAY WORKS

Protein Extraction from cells,
tissues, or bodily fluids



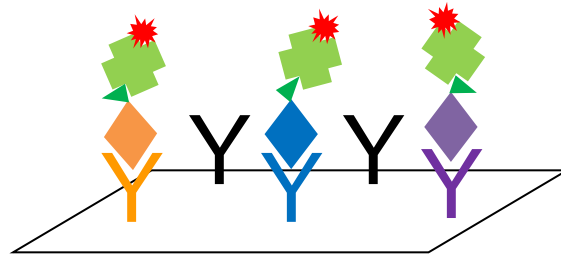
Protein Biotinylation



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 lysis buffer; 2) Biotinylate protein samples; 3) Incubate labeled samples with antibody array; and 4) Detection by dye conjugated streptavidin.

Suitable samples include cell lysate, tissue lysate, serum, plasma, culture supernatant, lysate from FFPE tissues.

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.

SAMPLE PREPARATION

The following sample formats are accepted. Please send all samples in 1.5 mL or 2.0 mL screw-cap tubes.

- **Cell pellet** – 5 million cells in frozen pellets
- **Cell lysate** – 400 ug or more total protein at a concentration of 2ug/uL or higher
 - **Important:** Only use PBS to wash media/cells. To protect protein activity, avoid using trypsin or other reagents. Cell lysis can be performed with commonly used lysis protocols. Acceptable lysis buffers include Full Moon BioSystems' Protein Extraction Buffer (Cat #: EXB050), RIPA Buffer from Pierce, or other commonly used buffers. Tris and detergents should be avoided or limited. No more than 50mM of Tris, 0.1% SDS, or 1% non-ionic detergents should be present in the sample. Protease and/or phosphatase inhibitors (such as Roche's inhibitor cocktail) may be added to lysis buffer to preserve protein activity.
 - **Adherent Cells:** Remove media and wash the culture with ice cold 1X PBS 3 – 5 times. Remove remaining PBS and add lysis buffer. Detach cells using a scraper and transfer the cells and remaining supernatant to a clean screw-top microtube. Proceed to cell lysis. For detailed lysis protocol, please refer to manufacturer's instructions for the lysis buffer used. Transfer final lysate to a clear tube.
 - **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 three times to ensure complete removal of media. Discard supernatant. Proceed to cell lysis. For detailed lysis protocol, please refer to manufacturer's instructions for the lysis buffer used. Transfer final lysate to a clear tube.
- **Frozen tissue** – 75 mg of frozen tissues
- **Tissue lysate** – 400 ug or more total protein at a concentration of 2ug/uL or higher
 - Wash tissues with cold 1X PBS (4°C) with vortexing. Remove and discard PBS. Repeat 3 – 5 times.

Important: Make sure blood is completely removed from the tissues. 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 be clear and colorless. Any blood left in the tissues will get into lysates and lead to high background on the arrays.
 - Tissue lysis can be performed with commonly used lysis protocols. Acceptable lysis

buffers include Full Moon BioSystems' Protein Extraction Buffer, RIPA Buffer from Pierce, or other commonly used buffers. The use of Tris and detergents should be avoided or limited. No more than 50mM of Tris, 0.1% SDS, or 1% non-ionic detergents should be present in the sample. Protease and/or phosphatase inhibitors (such as Roche's inhibitor cocktail) may be added to lysis buffer to preserve protein activity.

- **Serum or plasma** – 20 uL of serum/plasma

PACKAGING

All samples (cell pellets, tissues or protein extracts) should be stored and sent in tubes with screw caps. Mark each tube with clear identifications.



All samples must be shipped on dry ice.

Preparing Dry Ice Shipment – Please contact your carrier for detailed packaging and shipping guidelines.



A dry ice shipping container includes a Styrofoam insulating liner and a heavy cardboard outer container for overall protection. The inner container should have insulated walls at least 1.25 inches (30mm) thick.

- Shipping with dry ice is potentially dangerous, including explosion, suffocation and contact hazards. Packages must allow for release of carbon dioxide gas. The dry ice must not be sealed in a container with an airtight seal. The outer shipping container must be clearly labeled with a hazard class 9 label, UN 1845 on the vertical side of the container and must display the weight of the dry ice in kilograms.
- Dry ice will sublime from a solid to a gas at a rate of 5-10 pounds per 24 hours when shipped in an insulated cooler with lid. Therefore, when using dry ice for shipping, it is important add sufficient amount of dry ice to maintain the proper temperature throughout the entire transit time of the shipment.

Carefully pack your samples in the Styrofoam insulated container. Add dry ice. Close the lid. Place the insulated container inside the outer container; close and seal with packaging tapes. Clearly mark "Perishable" on the outside of the package.

SHIPPING

Ship the package using any carrier that accepts dry ice shipments. Be sure to drop it off before carrier's daily cut-off time to minimize transit time.

Delivery address:

Attn: Antibody Array Services
Full Moon BioSystems, Inc.
754 North Pastoria Avenue
Sunnyvale, CA 94085
U.S.A.
Phone: 408.737.1702
Email: support@fullmoonbio.com

To ensure your samples arrive at our facility in good order, use the following services to ship your package:

- Shipments originating in North America – Ship on any day from Monday to Wednesday. Do not ship on a Thursday or Friday.
 - FedEx: Priority Overnight or Standard Overnight
 - UPS: Next Day Air or Next Day Air Saver
- Shipments originating outside of North America – The best day to ship is on a Monday to ensure arrival in the US for delivery later the same week.
 - FedEx: International Priority
 - UPS: Worldwide Express
 - DHL: Worldwide Express

As soon as you ship the package, email your shipment tracking number to your contact person at Full Moon BioSystems or to support@fullmoonbio.com.

RESULTS

Signal intensity data is extracted from array images, organized and analyzed. Fold changes between samples are determined. Below is the protocol we use for analysis:

1. Median signal intensity for each spot is extracted from array image.
2. For each antibody, determine the average signal intensity for replicate spots.
3. Determine the coefficient of variation for replicate spots.

4. Normalize signal intensity data.
5. Determine fold change between samples.

- [Sample Assay Results](#)

CONTACT INFORMATION

Technical Support:

Tel: 408.737.2875

Email: support@fullmoonbio.com

Main Contact:

Tel: 408.737.1702

Fax: 408.732.7230

Email: info@fullmoonbio.com

Address:

Full Moon BioSystems, Inc.
754 North Pastoria Avenue
Sunnyvale, CA 94085

REQUIRED DOCUMENTATION

All shipments

- Sample Submission Form (Page 9 - 10)

International Shipments:

Material derived from any animal is potentially subject to U.S. Department of Agriculture (USDA) regulations and must be cleared by Department of Homeland Security, Customs and Border Protection (DHS, CBP) Agricultural Specialists at the port of arrival before entry into the United States is authorized. However, the USDA does not have regulatory authority over the importation of live laboratory animals or laboratory mammal material that have not been inoculated with or exposed to any livestock or poultry disease agents exotic to the United States.

Material derived from rodents and other small mammals which: (a) have not been inoculated with, or exposed to any exotic livestock or poultry disease agents, and (b) do not originate from

facilities where work with exotic disease agents affecting livestock or avian species is conducted, may be imported without USDA, APHIS, Veterinary Services (VS) restrictions.

The following documents are required for U.S. Customs clearance. Please place them on the outside of the package:

1. Shipper's Declaration (A sample declaration is provided on Page 11)
 - Include statements:
 - identifying a detailed description and quantity (uL or mg) of the material and naming the animal species;
 - confirming that the material was derived only from laboratory mammals that have not been inoculated with or exposed to any livestock or poultry disease agents exotic to the United States;
 - confirming that the material was derived only from laboratory mammals that did not originate from a facility where work with exotic disease agents affecting livestock or avian species is conducted, and
 - identifies the immunogen for antibodies/antiserum, if applicable.
 - Provide your detailed contact information in the document.
 - The declaration should be provided on your organization's letterhead containing the organization's physical address.
2. Commercial Invoice – 3 copies
 - Blank commercial invoice forms can be found on your carrier's website.
 - Use the following information to prepare your commercial invoice:
 - Item description: Description of your samples
 - Declared Value: \$10
 - Shipping Remarks: Contents have no commercial value. Value declared for customs purpose only.
3. Airway Bill
 - All transportation charges, duty and taxes, and fees must be paid by Sender. Shipments must be delivered duty paid (DDP). (Research samples are duty and tax free. Small processing fees may be assessed by USDA or FDA.)
 - Be sure to mark the correct billing option to avoid delays.
 - T/C (transportation charge): Sender
 - D/T (duty and taxes): Sender

Antibody Array Assay Service Sample Submission Form**Customer Information**

Company Name: _____

Contact Name: _____

Phone: _____

Email: _____

Payment Method: ☐ Purchase Order – PO#: _____
☐ Credit Card - Please call 408.737.2875 to provide card information.
☐ Prepayment - Payment date: _____

Sample Information

Number of Samples:	_____
Sample Information: (All sample information collected here will be used solely for antibody array assay optimization and will be kept confidential.)	<p>Cell type (e.g. human breast cancer cells): _____ <input type="checkbox"/> Cell pellets <input type="checkbox"/> Cell lysate, please provide lysis buffer components: _____ _____</p> <p>Tissue type (e.g. mouse heart tissue): _____ <input type="checkbox"/> Frozen tissues <input type="checkbox"/> Tissue lysate, please provide lysis buffer components: _____ _____</p> <p><input type="checkbox"/> Serum/Plasma</p> <p>Other sample type: _____</p> <p>Additional information: _____</p>
Sample Identification:	Please provide the information on page 2

Array(s) to be used: _____

All samples submitted were not originated from sources involving infectious diseases and did not involve any infectious inoculation. I hereby certify that all the information provided in this form is correct and accurate.

Name of the person completing this form: _____

Signature: _____ Date: _____

Continue on next page

Sample Identification

<u>Sample ID</u>	<u>Sample Quantity</u> (number of cells, weight of tissues, lysate concentration)

How would you like the assay data compared? (e.g. Sample 1 vs. Sample 2; Sample 3 vs. Sample 4)

Untreated Sample	Treated Sample

Additional Comments: _____

Be sure to send this form with your samples

Must Be Provided on Your Organization's Letterhead

SHIPPER'S DECLARATION

Date: _____

This package contains _____
Detailed description of the Material

The material was derived only from laboratory mammals that have not been inoculated with or exposed to any livestock or poultry disease agents exotic to the United States;

The material was derived only from laboratory mammals that did not originate from a facility where work with exotic disease agents affecting livestock or avian species is conducted;

The material is intended for biomedical laboratory research and analysis purposes only, not for human use.

The exporter of the material covered by this document declares that these products are of
_____ origin.
Country Name

Sincerely,

Signature: _____

Name: _____

Company Name: _____

Address: _____

Phone: _____

Email: _____