invitrogen™

ProtoArray® Human Protein Microarray v5.0 for Immune Response BioMarker Profiling (IRBP)

IRBP Experienced Users Guide

Introduction

This quick reference contains brief instructions for using the ProtoArray® Human Protein Microarray v5.0 for the immune response biomarker profiling (IRBP) application. It is intended for experienced users of ProtoArray® Microarrays.

If you are a first time user, refer to the **ProtoArray® Applications Guide** available at www.invitrogen.com, for detailed instructions for performing the IRBP application, microarray specifications, ProtoArray® technology overview, troubleshooting, and license information.

Experimental Overview

1. Block the ProtoArray® Human Protein Microarray with Blocking Buffer.
2. Probe the array with diluted (1:500) human serum or plasma.
3. Perform detection using Alexa Fluor® 647 goat anti-human IgG.
4. Dry the array for scanning.
5. Scan the array with a fluorescent microarray scanner to obtain an array image.
6. Download the protein array lot specific information from ProtoArray® Central portal and acquire the image data using microarray data acquisition software.
7. Analyze results using ProtoArray® Prospector data analysis software available from www.invitrogen.com/protoarray.

Important Guidelines

To obtain the best results with the ProtoArray® Human Protein Microarray, follow these guidelines:

- The ProtoArray® Microarray can only be used once. **Do not re-use or re-probe** the array.
- Always wear clean gloves while handling microarrays. Take the appropriate precautions (wear a laboratory coat, disposable gloves, and eye protection) when handling serum or plasma, and dispose of used samples as biohazardous waste.
- **Do not** touch the surface of the array. Damage to the array surface can result in uneven or high background.
- **Do not** use LifterSlip™ or any other coverslip for the IRBP application.
- Maintain the array and reagents at 2–8°C during the experiment.
- Avoid drying of the array during the experiment. Ensure the array is completely covered with the appropriate reagent during all steps of the protocol.
- Perform array experiments at a clean location to avoid dust or contamination. Filter solutions as needed (particles invisible to the eye can produce high background signals and cause irregular spot morphology).
- Dry the array by centrifugation prior to scanning. **Do not** dry the array using compressed air or commercial aerosol sprays. Scan the array immediately upon completion of the experiment.
- Avoid exposing the array to light after probing with a fluorescent detection reagent.
- Select the appropriate application from the **Mode Selection Menu** in ProtoArray® Prosepctor when performing analysis of microarray results. Use the **Immune Response Profiling with Plasma** application for plasma samples, and the **Immune Response Profiling** application for serum samples.

Continued on next page

Part no. 25-1011 MAN0000600 Rev. Date: 1 April 2011

Materials Needed

- Human serum or plasma sample (dilute the sample 1:500 in Washing Buffer, store on ice until use)
- ProtoArray® Human Protein Microarray v5.0 and buffers (see recipes below)
- Alexa Fluor® 647 Goat Anti-Human IgG (Invitrogen, Cat. no. A21445)
- 10X Synthetic Block (Invitrogen, Cat. no. PA017)
- Clean, 4-chamber incubation tray with cover (Sarstedt 94.6077.307), chilled on ice
- Forceps and deionized water
- Shaker (capable of circular shaking at 50 rpm, place the shaker at 4ºC)
- Microarray slide holder and centrifuge equipped with a plate holder (*Optional*)
- Fluorescence microarray scanner (refer to the ProtoArray® Applications Guide for recommended microarray scanners)
- Microarray data acquisition software (*e.g.* GenePix® Pro from Molecular Devices; refer to the ProtoArray® Applications Guide for details and optional software packages)
- Data analysis software (ProtoArray® Prospector recommended, available from www.invitrogen.com/protoarray)

Sample Preparation

The IRBP application has been optimized for use with human serum and plasma samples (fresh or frozen). Avoid repeated freeze-thaw cycles with samples. Prior to use, process the sample to remove any aggregates by centrifugation (12,000 × *g* for 30 seconds in a microcentrifuge), if needed.

We recommend using a 1:500 dilution of the serum or plasma sample in Washing Buffer to maximize signals while minimizing false positive and false negative results. Based on your initial results, you may need to optimize the serum dilution to obtain optimal performance.

Preparing Buffers

Prepare buffers **fresh** for best results. Mix buffers using the Blocking Buffer Kit (Invitrogen, Cat. no. PA055), or from scratch as described below. Mix stocks in a glass bottle. **Cool buffers to 4°C before use**.

Blocking Buffer*
(50 mM HEPES, pH 7.5, 200 mM NaCl, 0.08% Triton® X-100, 25% Glycerol, 20 mM Reduced glutathione, 1X Synthetic Block, 1 mM DTT)
5 ml buffer required per microarray.

1. Prepare 50 ml Blocking Buffer **fresh** as follows:

1 M HEPES, pH 7.5	2.5 ml
5 M NaCl	2 ml
10% Triton® X-100	0.4 ml
50% Glycerol	25 ml
Reduced glutathione	305 mg
10X Synthetic Block	5 ml
Deionized water	to 50 ml

2. Adjust pH to 7.5 with NaOH.
3. Mix reagents, chill to 4°C and add 50 µl of 1 M DTT prior to use.
4. Use buffer immediately. Store any remaining buffer at 4ºC for <24 hours.

Washing Buffer
(1X PBS, 0.1% Tween 20, 1X Synthetic Block)
60 ml buffer required per microarray.

1. Prepare 600 ml Washing Buffer **fresh** as follows:

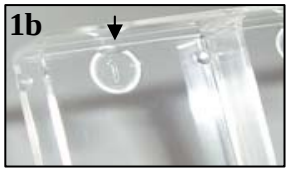
10X PBS	60 ml
10% Tween 20	6 ml
10X Synthetic Block	60 ml
Deionized water	to 600 ml

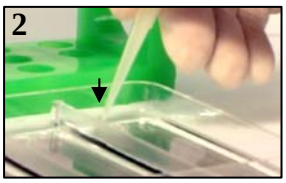
2. Mix reagents and cool to 4°C.
3. Use buffer immediately. Store any remaining buffer at 4ºC for <24 hours.

* Blocking Buffer without 10X Synthetic Block and DTT may be prepared the day before the assay. Store stock at 4°C for no more than 24 hrs.

Continued on next page

Blocking the Microarray





1. Remove the mailer containing the ProtoArray® Human Protein Microarray v5.0 from storage and place immediately at 4°C. Equilibrate the mailer at 4°C for at least 15 minutes prior to blocking. Not doing so may result in condensation on the array which can reduce protein activity or alter spot morphology.
2. Place one microarray with the barcode facing up into each well of a 4-chamber incubation tray (see previous page) such that the barcoded end of the microarray is near the end of the tray marked with an indented numeral (see figure 1a). The indentation in the tray bottom is used as the site for buffer removal (see figure 1b, arrow).
3. Using a sterile pipette, add 5 ml Blocking Buffer equilibrated to 4°C into each chamber with an array. **Avoid pipetting buffer directly onto the array surface.**
4. Incubate the tray for 1 hour at 4°C on a shaker set at 50 rpm (circular shaking).
5. After incubation, aspirate Blocking Buffer using vacuum or a pipette. Position the tip of the aspirator or pipette into the indentation at the end of the tray (see figure 1b, arrow) and aspirate the buffer from each well (see figure 2). Tilt the tray so that any remaining buffer accumulates at the base of the well at the numbered end of the tray and aspirate.

   Important: Do not position the tip on, or aspirate from the microarray surface as this can cause scratches. Immediately proceed to adding the next solution to prevent any part of the array surface from drying.
6. Proceed immediately to the **Probing the Microarray**.

Probing the Microarray

1. Add 5 ml Washing Buffer at the numbered end of the 4-chamber incubation tray without touching the array surface. Incubate the tray for 5 minutes at 4ºC on a shaker set at 50 rpm (circular shaking).
2. Aspirate the buffer using vacuum or pipette as described under **Blocking the Microarray** (Step 5).
3. Add 5 ml serum or plasma sample diluted (1:500) in Washing Buffer at the numbered end of the 4-chamber incubation tray without touching the array surface. Allow the sample to flow across the array surface. **Avoid pipetting sample directly onto the array surface.**
4. Incubate the tray for 90 minutes at 4ºC on a shaker set at 50 rpm (circular shaking).
5. Aspirate the sample using vacuum or pipette as described under **Blocking the Microarray** (Step 5).
6. Wash each array with 5 ml Washing Buffer with gentle shaking on a shaker set at 50 rpm for 5 minutes at 4ºC. Aspirate the Washing Buffer as described under **Blocking the Microarray** (Step 5).
7. Repeat wash step four more times using fresh Washing Buffer each time to obtain a total of 5 washes.
8. During the wash steps, mix 2.5 µl Alexa Fluor® 647 goat anti-human IgG antibody with 5 ml Washing Buffer per array to obtain a final antibody concentration of 1 µg/ml. Store on ice until use. Optional: if using exogenous anti-V5 antibody for serum-independent normalization, see detailed instructions in the **ProtoArray® Applications Guide**.
9. Add 5 ml Alexa Fluor® 647 antibody solution from Step 8 to the incubation tray at the numbered end of the tray without touching the array surface. Allow the solution to flow across the array surface. **Avoid pipetting solution directly onto the array surface.**
10. Incubate the tray for 90 minutes at 4ºC on a shaker set at 50 rpm (circular shaking).
11. Aspirate the antibody solution as described under **Blocking the Microarray** (Step 5).
12. Wash each array with 5 ml Washing Buffer with gentle shaking on a shaker set at 50 rpm for 5 minutes at 4ºC. Aspirate the Washing Buffer as described under **Blocking the Microarray** (Step 5).
13. Repeat wash step four more times using fresh Washing Buffer each time to obtain a total of 5 washes.
14. Proceed immediately to **Drying and Scanning the Microarray**, next page.

Continued on next page

Drying and Scanning the Microarray

1. To remove array from the 4-chamber incubation tray, insert the tip of the forceps into the indentation at the numbered end of the tray and gently pry the array upward (see figure 3). Using a gloved hand, pick up the array by holding the array by its **edges** only.
2. Insert the array into a slide holder and quickly rinse by submerging array into a large beaker filled with deionized water one time. Ensure the array is properly placed and is secure in the holder to prevent damage to the array during centrifugation.
3. Immediately centrifuge the array in the slide holder or 50 ml conical tube at 200 × *g* for 1 minute in a centrifuge (equipped with a plate rotor, if you are using the slide holder) at room temperature. Ensure the array is completely dry.
4. After drying, store the arrays vertically or horizontally in a slide box **protected from light** and avoid prolonged exposure to light. To obtain the best results, scan the array within 24 hours of probing.
5. To scan the array, start the appropriate array acquisition and analysis software on the computer connected to the fluorescence microarray scanner.
6. Insert the array into the fluorescent microarray scanner such that the nitrocellulose-coated side faces the laser source and barcode on the array is closest to the outside of the instrument.
7. Adjust scanner settings as follows:
   - Wavelength: 635 nm
   - PMT Gain: 600
   - Laser Power: 100%
   - Pixel Size: 10 µm
   - Lines to Average: 1.0
   - Focus Position: 0 µm
8. Preview the microarray. Adjust PMT Gain, if needed. Scan the microarray in detail and include the barcode for your records.
9. Save the image to a suitable location as 'multi-image TIFF' file. Remove the microarray from the scanner.
10. Proceed to **Data Acquisition and Analysis**, below.

Data Acquisition and Analysis

1. Connect to the portal at www.invitrogen.com/protoarray and then click on the **ProtoArray® Lot Specific Information** link that can be found under **BioMarker Discovery Resources**.
2. Enter the array barcode in the **Input Barcode Number** box and click on the **Search** button.
3. For each input barcode, various lot specific files are displayed.
4. Start the GenePix® Pro microarray data acquisition software on the computer. Open the saved image (.tiff) from Step 9 (**Drying and Scanning the Microarray**) and open the .GAL files downloaded from ProtoArray® Central. The .GAL file defines the array grid required by the data acquisition software.

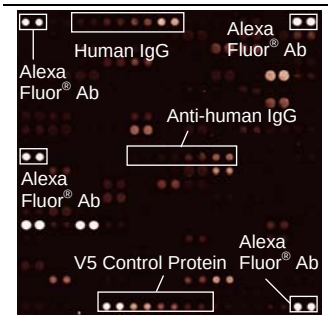
   Important: Make sure you download the latest files for the specific barcode on your array. Lot specific information files are updated frequently based on recently available sequence or protein information.
5. Adjust the subarray grid to ensure the grid is in proper location for each subarray. After the grid is properly adjusted and all features are aligned, acquire the pixel intensity data for each feature by clicking the **Analyze** button in GenePix® Pro, and save/export the results as a .GPR (GenePix® Results) file.
6. Use the files from Step 5, above, for data analysis using ProtoArray® Prospector (available through the **Online Tools** link under **BioMarker Discovery Resources** at www.invitrogen.com/protoarray).
7. Install ProtoArray® Prospector on your computer.
8. Start ProtoArray® Prospector from the desktop icon. Set the **Application** to Immune Response Profiling (for serum samples), or Immune Response Profiling with Plasma (for plasma samples).
9. Select the **Analyze** button from the Tool Bar.
10. Select the .GPR files from the "Files of type" pull-down list and navigate to your data file(s). Select the file(s) for analysis and click the **Open** button.
11. After analysis, ProtoArray® Prospector generates a list of human proteins showing significant interactions with the sample. The proteins that score as positive, are proteins that satisfy the basic program options. We recommend validating the interactions as described in the ProtoArray® Applications Guide.

Continued on next page

Expected Results

Use controls printed on the ProtoArray® Human Protein Microarray v5.0 in verifying the probing, detection, and scanning protocols. Refer to the ProtoArray® Applications Guide for details.

An example of control spots obtained with the ProtoArray® Human Protein Microarray v5.0 probed with 1:500 diluted human serum and Alexa Fluor® 647 goat anti-human IgG antibody are shown below.

Image	Control	Description/Function	Verification
	Alexa Fluor® antibody signal	Alexa Fluor® labeled antibodies serve as a positive control for fluorescence scanning and for orientation of the microarray image.	Alexa Fluor® antibody signal
	BioEase™ (biotin) V5 control protein signal	A positive control with biotin and V5 tags for detection with an anti-V5-Alexa Fluor® 647 antibody and Alexa Fluor® 647 conjugated streptavidin.	BioEase™ (biotin) V5 control protein signal
	Human IgG	A protein gradient of purified human IgG is printed on each subarray. Serves as a positive control when anti-human IgG is used for detection.	Proper probing, detection reagents, and serves as a positive control for IRBP application.
	Anti-human IgG	A protein gradient of goat anti-human IgG is printed on each subarray. The IgG from human serum binds to the anti-human IgG on the array and serves as a positive control.	Proper probing, detection reagents, and serves as a positive control for IRBP application.

Additional Products

The table below lists additional products available separately from Invitrogen. For more information about these products, visit www.invitrogen.com or contact Technical Support.

Product	Quantity	Catalog no.
ProtoArray® Products		
ProtoArray® Human Protein Microarray v5.0	1 array 20 arrays	PAH052501 PAH0525020
ProtoArray® Control Protein Microarray v5.0	1 array	PA10057
10X Synthetic Block	75 ml	PA017
Blocking Buffer Kit	1 kit	PA055
Array Control Protein	40 µl	451096
Alexa Fluor® 647 Goat Anti-Human IgG	500 µl	A21445
Phosphate Buffered Saline (PBS), 1X	500 ml	10010-023