ProtoArray® Human Protein Microarray v5.0 for for Protein-Protein Interaction (PPI)

PPI Experienced Users Guide

This quick reference contains brief instructions for using the ProtoArray® Human Protein Microarray v5.0 for the protein-protein interacion (PPI) application, to identify interactions between a protein probe of interest and human proteins printed on the array using antibody or streptavidin based fluorescence detection methods. 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 PPI application, microarray specifications, ProtoArray® technology overview, troubleshooting, and license information.

Experimental Overview

1. Block the ProtoArray® Human Protein Microarray with Blocking Buffer.
2. Probe array with protein probe in Washing Buffer and detect by fluorescent conjugated primary or secondary detection method.
3. Dry the array for imaging.
4. Scan the array with a fluorescent microarray scanner to obtain an array image.
5. Download the protein array lot specific information from ProtoArray® Central portal and acquire the image data using microarray data acquisition software.
6. Analyze results with ProtoArray® Prospector data analysis software available at ww.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.
- Use Alexa Fluor® 647 or Cy5™ dyes for detection on the ProtoArray® Human Protein Microarray. Alexa Fluor® 555 or Cy3™ dyes can result in higher background.
- Always wear clean gloves while handling microarrays.
- **Do not** touch the surface of the array. Damage to the array surface can result in uneven or high background.
- 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.

Protein Probe Requirements

We recommend using protein probes at a concentration of 100 nM–10 µM if biotinylated, and 10 nM–1 µM if V5 epitope-tagged. Protein probes are diluted in Casein Washing Buffer (see recipe on page 2) to a final volume of 120 µl. If you purify your own protein of interest, observe the following guidelines:

- **Purify the protein under native conditions.**
- Proteins should be > 90% pure as determined by Coomassie® staining.
- Perform an activity assay of the protein after purification using your method of choice.
- Make sure the protein is soluble and active in buffers used for probing the microarray (see page 2).

Intended Use

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

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Materials Needed

- ProtoArray® Human Protein Microarray v5.0 and buffers (see recipes below)
- Purified protein of interest (see above for requirements); store on ice until use
- Anti-V5 Antibody (Invitrogen, Cat. no. R960-25) or an appropriate primary antibody against your protein probe of interest
- Alexa Fluor® 647 Goat Anti-Mouse IgG (Invitrogen, Cat. no. A21236) or Goat Anti-Rabbit IgG (Invitrogen, Cat. no. A21245)
- Streptavidin-Alexa Fluor® 647 Conjugate (Invitrogen, Cat. no. S-32357) or Alexa Fluor® 647 Anti-V5 Antibody for ProtoArray® (Invitrogen, Cat. no. 451098)
- Clean, 4-chamber incubation tray with cover (Greiner, Cat. no. 96077307 or ISC Bioexpress, Cat. no. T-2896-1), chilled on ice
- Forceps and deionized water
- LifterSlip™ (Thermo Scientific, Cat. no. 25x60I-2-4789)
- 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 available at www.invitrogen.com/protoarray, recommended)

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.

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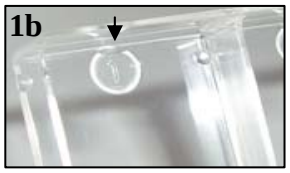
Preparing Antibody/Streptavidin Solution

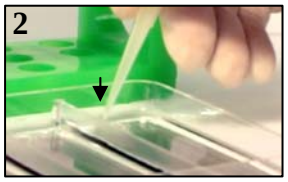
The protein probe is detected using a primary or secondary fluorescent conjugate. Any primary antibody specific to the protein probe can be used for detection, but optimal conditions may need to be independently developed. Primary antibodies can be labeled using the Alexa Fluor® 647 Protein Labeling Kit (Invitrogen, Cat. no. A-20173). Prepare 5 mL of antibody or streptavidin solution for each array to be probed.

- **Primary biotin detection**: Prepare 1 µg/mL Streptavidin-Alexa Fluor® 647 Conjugate in Washing Buffer
- **Primary V5-epitope detection**: Prepare 1 µg/mL Alexa Fluor® 647 Anti-V5 Antibody in Washing Buffer
- **Secondary V5-epitope detection**:
  - Use 1 µg/mL Anti-V5 Antibody in Washing Buffer for primary antibody
  - Use 1 µg/mL Alexa Fluor® 647 Goat Anti-Mouse diluted to 1 µg/mL in Washing Buffer for secondary antibody

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 chilled 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**.





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Probing the Microarray

1. Remove array from 4-well tray by inserting the tip of forceps into the indentation at the numbered end of the tray and gently prying the array upward (see figure 3). Pick up array with a gloved hand taking care to only touch the array by its edges. Gently dry the back and sides of the array on a paper towel to remove excess buffer.

   Note: To ensure that the array surface remains wet, do not dry more than 2 arrays at a time before adding the diluted protein and LifterSlip™.
2. Pipet 120 µL of protein probe in Washing Buffer on top of the array, then place a LifterSlip™ over the printed area of the array using forceps, as shown below. The **raised edges of the LifterSlip™ should face the surface of the array**. If air bubbles are observed under the LifterSlip™ gently raise the LifterSlip™ and slowly lower it again.

3. Incubate for 90 minutes at 4ºC keeping the 4-well tray flat with the arrays facing up (no shaking).
4. Add 5 mL fresh Washing Buffer, and remove the LifterSlip™ with forceps, taking care not to scratch the array surface with the LifterSlip™ or forceps. Wash for 5 minutes with gentle agitation. Remove Washing Buffer by aspiration (see Step 5 of **Blocking Procedure** for details).
5. Repeat wash step four more times.
6. Add 5 mL of primary antibody or Alexa Fluor® 647 conjugate (see **Preparing Antibody/Streptavidin Solution**)

   Note: Always add diluted antibody at the numbered end of the 4-well tray, allowing the liquid to flow across the array surface. **Avoid direct contact with the array** and if at all possible, avoid applying the antibody solution directly onto the array.
7. Incubate for 90 minutes at 4ºC with gentle circular shaking (~50 rpm).
8. Remove solution by aspiration (see **Blocking Procedure**).
9. Wash with 5 mL fresh Washing Buffer for 5 minutes with gentle agitation. Remove Washing Buffer by aspiration (see **Blocking Procedure**).
10. Repeat wash step four more times.
11. Add 5 mL of Alexa Fluor® 647 conjugated secondary antibody diluted in Washing Buffer (if necessary).

    Note: This step is not needed if performing detection using a labeled primary antibody or Streptavidin-Alexa Fluor® 647 Conjugate.
12. Incubate for 90 minutes at 4ºC with gentle circular shaking (~50 rpm).
13. Remove secondary antibody by aspiration (see **Blocking Procedure**).
14. Wash with 5 mL fresh Washing Buffer for 5 minutes with gentle agitation. Remove Washing Buffer by aspiration (see **Blocking Procedure**).
15. Repeat wash step four more times.
16. Remove the array from the 4-well tray using forceps (see Step 1). Proceed to **Drying and Scanning the Microarray**.

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Drying and Scanning the Microarray

1. Insert array into a slide holder and quickly rinse by submerging into a large beaker filled with deionized water three times. Ensure the array is properly placed and is secure in the holder to prevent any damage to the array during centrifugation.
2. Dry the ProtoArray® Human Protein Microarray v5.0 by centrifugation. Spin the array at 200 × g for 1–2 minutes at room temperature in the slide holder (if using a centrifuge equipped with a plate rotor) or 50 mL conical tube (if using a swinging bucket rotor). Verify that the array is completely dry.
3. After drying, store the array vertically or horizontally in a slide box **protected from light**. Avoid prolonged exposure to light. To obtain the best results, scan the array within 24 hours of probing.
4. To scan the array, start the appropriate array acquisition and analysis software on the computer connected to the fluorescence microarray scanner.
5. Insert the array into the scanner such that the printed array surface faces the laser source and the barcode on the array is closest to the outside of the instrument.
6. 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

7. Preview the microarray. Adjust PMT Gain, if needed. Scan the microarray in detail and include the barcode for your records.
8. Save the image to a suitable location as 'multi-image TIFF' file. Remove the microarray from the scanner.
9. 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. Several lot specific files will be displayed for each input barcode. Download the .Gal file to your computer.
4. Start the GenePix® Pro microarray data acquisition software on the computer. Open the saved image (.tiff) from Step 8, above and open the .GAL file downloaded from ProtoArray® Central for protein arrays. The .GAL file defines the array grid required by the microarray data acquisition software.

   Important: Make sure you download files that are associated with the specific barcode on your array. Lot specific information files are updated frequently based on recent sequence or protein information, so download the latest version of lot specific information files.
5. Adjust the subarray grid to ensure the grid is in the 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 that can be found under **BioMarker Discovery Resources** at www.invitrogen.com/protoarray).
7. Install ProtoArray® Prospector.
8. Start ProtoArray® Prospector from the desktop icon. Set the **Application** to Protein-Protein Interaction.
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. After analysis, ProtoArray® Prospector generates a list of positive interactions with the ProtoArray® Human Protein Microarray v5.0 for PPI.

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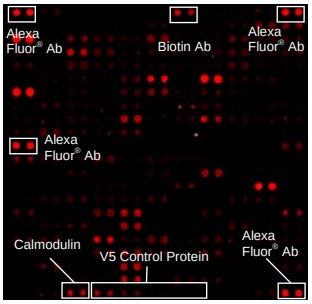
Expected Results

An example of spots obtained with the ProtoArray® Human Protein Microarray v5.0 after probing with 50 µg/mL of the Array Control Protein (*i.e.* BioEase™ -V5 tagged biotinylated calmodulin kinase) and Streptavidin-Alexa Fluor® 647 Conjugate are shown below. Refer to the ProtoArray® Applications Guide for additional details on control features.

Image	Control	Description/Function
	Alexa Fluor® antibody signal	Alexa Fluor® labeled antibodies printed on each subarray serve as a positive control for fluorescence scanning and for orientation of the microarray image.
	Biotin antibody signal	A mouse anti-biotin antibody serves as a positive control for biotinylated probes and anti-mouse antibodies.
	BioEase™ (biotin) V5 control protein signal	A biotin and V5 tagged control protein printed on the array for positive detection with anti-V5-Alexa Fluor® 647 antibody and Streptavidin-Alexa Fluor® 647 Conjugate.
	Calmodulin from yeast (Cmd1p) or human (CALM2)	A positive control for the protein-protein interaction application through activity between calmodulin printed on the array and the Array Control Protein. Refer to the lot specific .GAL file for the specific identity of the protein.

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 Anti-V5 Antibody for ProtoArray®	80 µl	451098
ProtoArray® Human Protein Microarray v5.0 PPI Kit *for V5-tagged proteins*	1 kit	PAH0525013
ProtoArray® Human Protein Microarray v5.0 PPI Kit *for biotinylated proteins*	1 kit	PAH0525011
Streptavidin-Alexa Fluor® 647 Conjugate (2 mg/mL)	0.5 mL	S-32357
Phosphate Buffered Saline (PBS), 1X	500 mL	10010-023