invitrogen™

ProtoArray® Human Protein Microarray v4.1 Protein-Protein Interaction (PPI) Kit

for biotinylated proteins

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User Manual

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Kit Contents and Storage

Shipping and Storage

The components included in the ProtoArray® Human Protein Microarray v4.1 PPI Kit *for biotinylated proteins* are shipped as detailed below. Upon receipt, store as indicated.

All kit components are stable for 12 months when stored properly. The **expiration date** is printed on the package for each array. Use the array before the expiration date for best results.

Components	Shipping	Storage
ProtoArray® Human Protein Microarray v4.1	Blue ice	–20°C
ProtoArray® Control Protein Microarray v4.1	Blue ice	–20°C
Array Control Protein	Dry ice	–20°C
Streptavidin-Alexa Fluor® 647 Conjugate	Blue ice	4°C
ProtoArray® PPI Buffer Module A	Dry ice	–20°C
ProtoArray® PPI Buffer Module B	Blue ice	4°C

ProtoArray® Human Microarray

Each ProtoArray® Human Protein Microarray v4.1 PPI Kit *for biotinylated proteins* contains mailers with **two** ProtoArray® Human Protein Microarrays v4.1.

Store the microarrays at –20°C.

For details on array specifications, see pages 5-8.

ProtoArray® Control Reagents

Each ProtoArray® Human Protein Microarray v4.1 PPI Kit *for biotinylated proteins* includes the following control reagents. **Store the microarray and Array Control Protein at –20°C.**

Item	Composition	Amount
ProtoArray® Control Protein Microarray v4.1	--	2 arrays
Array Control Protein (biotinylated calmodulin kinase with a V5 tag)	0.5 mg/ml in phosphate-buffered saline (PBS), pH 7.4	40 µl

For details on array specifications, see page 8. For information about the Array Control Protein, see page 16.

Continued on next page

Streptavidin-Alexa Fluor® 647 Conjugate

The ProtoArray® Human Protein Microarray v4.1 PPI Kit *for biotinylated proteins* contains one tube of Streptavidin-Alexa Fluor® 647 Conjugate with the following specifications:

- **Concentration:** 2 mg/ml in phosphate-buffered saline (PBS), pH 7.2 with 5 mM sodium azide
- **Amount supplied:** 30 µl

Store at 4°C. Protect the Streptavidin-Alexa Fluor® 647 Conjugate from exposure to light.

ProtoArray® PPI Buffer Module A

The ProtoArray® PPI Buffer Module A includes the following reagents.

Store at –20°C.

Note: The amount of reagents supplied is sufficient to perform 4 microarray screening experiments.

Item	Composition	Amount
ProtoArray® Casein Blocking Buffer	100 mM sodium phosphate, pH7.5, 200 mM NaCl, 0.08% Triton X-100, 25% glycerol, 20 mM reduced glutathione, 1% Hammersten grade casein	20 ml
ProtoArray® Casein Washing Buffer	1x PBS, pH7.4, 1% Hammersten grade casein, 0.1% Tween 20	240 ml
DTT	1 M DTT in deionized water	400 µl

ProtoArray® PPI Buffer Module B

The ProtoArray® PPI Buffer Module B includes the following reagents.

Store at room temperature.

Item	Composition	Amount
LifterSlip™ Coverslip	60 mm x 22 mm, RNase-free	5 coverslips per pack
4-Chamber Incubation Tray	--	1

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Accessory Products

Additional Products

The table below lists additional products available separately from Invitrogen. For more information about these products, visit www.invitrogen.com or call Technical Support (page 38).

Product	Quantity	Catalog no.
ProtoArray® Human Protein Microarray v4.1 for KSI	1 array	PAH0524106
ProtoArray® Control Protein Microarray v4.1 for KSI	1 array	PA10012
ProtoArray® Human Protein Microarray v4.1 for PPI	1 array	PAH0524101
ProtoArray® Control Protein Microarray v4.1 for PPI	1 array	PA10017
ProtoArray® Human Protein Microarray v4.1 for IRBP	1 array	PAH0524102
ProtoArray® Human Protein Microarray v4.1 PPI Complete Kit *for epitope-tagged proteins*	1 kit	PAH05241013
ProtoArray® Human Protein Microarray v4.1 KSI Complete Kit *for kinase substrate identification*	1 kit	PAH05241065
Biotin-XX Microscale Protein Labeling Kit and FluoReporter® Biotin Quantitation Assay Kit	1 kit	B30756
ProQuest™ Two-Hybrid System	1 kit	PQ10002-01
ProQuest™ Two-Hybrid System with Gateway® Technology	1 kit	PQ10001-01
NuPAGE® Novex® 4-12% Bis-Tris Gel (1.0 mm, 10-well)	1 box	NP0321BOX
NuPAGE® MOPS SDS Running Buffer (20X)	500 ml	NP0001
NuPAGE® MES SDS Running Buffer (20X)	500 ml	NP0002
NuPAGE® Sample Reducing Agent (10X)	250 µl	NP0004
NuPAGE® Antioxidant	15 ml	NP0005
NuPAGE® LDS Sample Buffer (4X)	10 ml	NP0007

Pre-Cast Gels and Pre-made Buffers

A variety of pre-cast gels including NuPAGE® Novex® Pre-cast Gels and pre-made buffers for gel electrophoresis are available from Invitrogen. For details on these products, visit our website at www.invitrogen.com or contact Technical Support (page 38).

Introduction

Overview

Introduction

The ProtoArray® Human Protein Microarray v4.1 Protein-Protein Interaction (PPI) Kit *for biotinylated proteins* supports rapid and efficient detection of human protein-protein interactions using a biotinylated protein of interest supplied by the user to probe a ProtoArray® Human Protein Microarray v4.1. Each array contains thousands of purified human proteins printed in duplicate on a nitrocellulose coated glass slide. See below for an overview of the system.

The ProtoArray® Human Protein Microarray v4.1 PPI Kit does not include a module for biotinylation of the protein probe. See previous page for additional products necessary to perform biotinylation of protein probes.

ProtoArray® Microarray PPI Applications

ProtoArray® Human Protein Microarray allow you to:

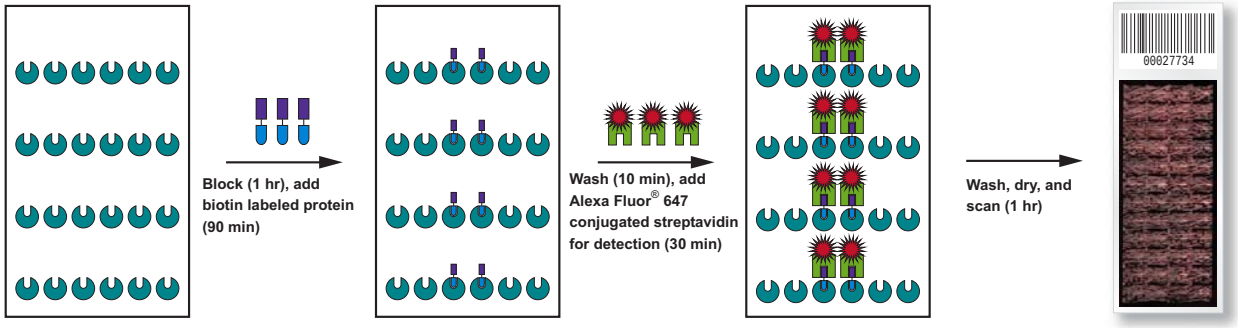
- Detect novel protein-protein interactions
- Validate previously observed protein-protein interactions
- Confirm positive interactions using the identified interacting protein on the array as a probe in reciprocal experiments
- Test various experimental conditions for your protein-protein interactions

System Overview

To use the ProtoArray® Human Protein Microarray PPI Kit, you will:

- *In vitro* biotinylate your protein of interest using your biotinylation kit of choice
- Use the biotinylated protein to probe the ProtoArray® Control Protein Microarray to verify protein biotinylation and probing conditions.
- Probe the ProtoArray® Human Protein Microarray with the biotinylated protein probe to detect protein-protein interactions.

The ProtoArray® detection protocol includes instructions to block the array, probe the array with your biotinylated protein probe, wash to minimize non-specific interactions, detect interactions using the Streptavidin-Alexa Fluor® 647 Conjugate, dry, scan the array to view results, acquire the array image, and analyze results (see figure below). For a detailed experimental workflow, see page 12.



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Advantages

Using the ProtoArray® Human Protein Microarray PPI Kit to detect protein-protein interactions offers the following advantages:

- Provides a simple, rapid, and efficient method to identify protein interactions within a day
- Includes qualified buffers and detection reagents for probing, eliminating the need to prepare reagents
- Allows screening of your protein of interest against thousands of human proteins
- Provides sensitive, stable, fluorescence detection using the Alexa Fluor® 647 dye
- Built-in controls are printed on each array to control for background and detection
- Arrays are compatible with most commercially available fluorescence microarray scanners

Important

- Since most of the human proteins printed on the microarray contain a GST (Glutathione-S-Transferase) fusion tag and some proteins also contain a polyhistidine (6x) tag, **do not** use an anti-GST antibody or anti-polyhistidine antibody for detecting interactions on a ProtoArray® Human Protein Microarray. We strongly recommend that you probe the ProtoArray® Human Protein Microarray with only your detection reagent to detect signals resulting due to interactions between the detection reagent and proteins printed on the array.
- We recommend the use of Alexa Fluor® 647 dyes. Although Alexa Fluor® 555 or Cy3™ dyes can also be used, these dyes result in higher background signals.

Purpose of the Manual

This manual provides the following information:

- An overview of the ProtoArray® Human Protein and Control Protein Microarrays
- Instructions to probe the ProtoArray® Microarray with your protein probe
- Guidelines to perform data analysis
- Expected Results and Troubleshooting

Description of Kit Components

Components of the ProtoArray® PPI Kit *for biotinylated proteins*

The ProtoArray® Human Protein Microarray PPI Kit *for biotinylated proteins* include the following major components:

- The ProtoArray® Human Protein Microarray; a high-density protein microarray that allows you to screen your protein of interest (protein probe) against thousands of human proteins
- The ProtoArray® Control Protein Microarray and the Array Control Protein for verification of the probing conditions and background levels
- The ProtoArray® PPI Buffer Modules A and B contain pre-made, qualified reagents for performing the blocking and washing steps during probing
- The Streptavidin-Alexa® Fluor 647 Conjugate for detection

ProtoArray® Human Protein Microarray

The ProtoArray® Human Protein Microarrays are high-density protein microarrays containing human proteins. The ProtoArray® technology is based on the yeast protein microarray technology developed by Zhu *et al*., 2001 to detect molecular interactions with proteins.

Each human open reading frame (ORF) is expressed as an N-terminal GST (Glutathione-S-Transferase) fusion protein, purified, and printed in duplicate on a nitrocellulose-coated glass slide. The use of nitrocellulose as a surface to print the arrays ensures maximum protein assay performance since the nitrocellulose surface is known to be compatible with a variety of protein functions (Espejo *et al.*, 2002; Kukar *et al.*, 2002; Michaud *et al.*, 2003). The nitrocellulose coating is thin and does not interfere with scanning of the array.

Each ProtoArray® Protein Microarray PPI Kit *for biotinylated proteins* includes two microarrays to allow you to assay for protein interactions using different experimental conditions or two distinct proteins. Using a labeled protein probe, you can screen against the human proteins within a day to identify protein-protein interactions.

For array specifications and more details on how the human proteins are prepared, see pages 5-8.

ProtoArray® Control Protein Microarray

The ProtoArray® Control Protein Microarray contains various controls printed on a nitrocellulose-coated glass slide, and is used to validate the biotinylation and probing procedure **prior** to probing the ProtoArray® Human Protein Microarray.

Two control arrays are included in each kit; probe one array with your biotinylated protein probe to allow you to assess biotinylation quality, and probe the second array with the Array Control Protein supplied in the kit (biotinylated calmodulin kinase) to validate assay conditions and demonstrate a known protein-protein interaction between calmodulin kinase and yeast calmodulin (Cmd1p-Ybr109C).

For specifications and more details on the ProtoArray® Control Protein Microarray, see page 8.

Continued on next page

ProtoArray® PPI Buffer Module

The ProtoArray® PPI Buffer Module A and B include qualified reagents used in the blocking, washing, and detection steps during probing of ProtoArray® Microarrays. The pre-made buffers provide consistent results and eliminate the time required to prepare reagents.

ProtoArray® PPI Buffers Module B includes LifterSlip™ coverslips that hold a small reagent volume to minimize the amount of valuable probe used and prevent evaporation of reagents. Incubation Trays are also included in the module for blocking and washing the microarrays.

Alexa Fluor® 647 Detection

The high sensitivity, low background, signal stability, and commercial availability of fluorescence microarray scanners make fluorescence detection the preferred method for detecting protein-protein interactions on microarrays.

The ProtoArray® Human Protein Microarray PPI Kit *for biotinylated proteins* include the Streptavidin-Alexa Fluor® 647 Conjugate for detection of the biotinylated protein probe. The Alexa Fluor® 647 fluorophore is brighter and more stable than other commercially available dyes such as Cy™ Dyes and is more sensitive for detecting interactions on protein arrays. We have demonstrated that detection with Alexa Fluor® 647 produces approximately 2-fold higher signal/background ratios than Cy5™ detection.

ProtoArray® Central Portal

The ProtoArray® Central Portal at www.invitrogen.com/protoarray provides a web-based user interface to access ProtoArray® specific information including online tools, applications, and other resources. You will also use the portal to retrieve ProtoArray® Lot Specific Information (see page 28), which is required for analysis of the array data and identification of statistically significant interactions.

ProtoArray® Prospector

The ProtoArray® Prospector software quickly analyzes the microarray data acquired from the image acquisition software and easily identifies significant hits, saving you time and effort. In addition, the software has features that allow you to modify the analysis method and compare data obtained from different microarrays.

The ProtoArray® Prospector software and manual are available free-of-charge to ProtoArray® users, and are accessible online at the ProtoArray® Central Portal. To download the ProtoArray® Prospector software or manual, go to www.invitrogen.com/protoarray, and click on the **Online Tools** link under **BioMarker Discovery Resources**.

ProtoArray® Human Protein Microarray

Introduction

The ProtoArray® Human Protein Microarray v4.1 is a high-density protein microarray containing thousands of human proteins. Each human open reading frame (ORF) is expressed as an N-terminal GST fusion protein, purified, and printed in duplicate on a nitrocellulose-coated glass slide. This section provides details about the human protein microarray including array specifications and preparation of proteins.

Note: The ProtoArray® Human Protein Microarray PPI Kit *for biotinylated proteins* includes 2 ProtoArray® Human Protein Microarrays.

Human Protein Microarray Specifications

The specifications for the ProtoArray® Human Protein Microarray v4.1 are listed below.

Dimensions:	1 inch x 3 inch (25 mm x 75 mm)
Material:	Glass slide coated with a thin layer of nitrocellulose

The nitrocellulose-coated slide is from GenTel® BioSciences, Inc., and is specifically selected for superior performance of the array in the KSI application. APiX™ slides are manufactured by GenTel® BioSciences, Inc. APiX™ technology is covered by US Patent #6,861,251. APiX™ and Gentel® are registered trademarks of GenTel® Biosciences, Inc.

Each microarray has a barcode for tracking samples. The barcode number is also used to retrieve array specific information from the ProtoArray® Central Portal (see page 29).

Array Specifications

The array specifications for the ProtoArray® Human Protein Microarray are listed below.

The proteins on the microarray are printed in 48 subarrays and are equally spaced in vertical and horizontal directions.

For details on the subarray layout, and human protein and control spots on the ProtoArray® Human Protein Microarray, go to the ProtoArray® Central Portal at www.invitrogen.com/protoarray.

Total Subarrays:	48 (4 columns x 12 rows)
Subarray Size:	4400 µm x 4400 µm
Subarray Dimensions:	**20 rows x 22 columns**
Median Spot Diameter:	~110 µm
Spot Center to Center Spacing:	200 µm
Distance Between Subarrays:	100 µm
Replicates per Sample:	2
Total Human Proteins on v4.0 array:	~8300*

*Refer to ProtoArray® Central Portal for exact number of human proteins printed on the microarray.

Continued on next page

ProtoArray® Human Protein Microarray, continued

Array Content

The majority of the human protein collection is derived from the human Ultimate™ ORF (open reading frame) Clone Collection available from Invitrogen (see http://orf.invitrogen.com for more information). Each Ultimate™ ORF Clone is full insert sequenced and is guaranteed to match the corresponding GenBank® amino acid sequence.

Some of the human proteins printed on the array represent the human protein kinase collection derived from full insert sequenced clones but are not Ultimate™ ORF Clones. Some of the kinases from the kinase collection have been cloned as catalytic domains rather than full-length proteins. About 260 proteins printed on the array are derived from the purified protein kinase collection available from Invitrogen. Approximately 40 additional proteins printed on the array are purified cytokines available from Invitrogen. Approximately 25 proteins, peptides, and nucleic acids that have been demonstrated to be antigens in a variety of autoimmune diseases are also printed on the array.

For accession number and amino acid sequence for each protein as well as information on peptides and nucleic acids printed on the array, download the Protein Content List from www.invitrogen.com/protoarray.

Expression and Purification of Human Proteins

Almost all clones used to generate the human protein collection are entry clones consisting of a human ORF cloned into a Gateway® entry vector. Each entry clone is subjected to an LR recombination reaction with a Gateway® destination vector to generate an expression clone. The expression clone is then used to express the protein (as an N-terminus GST-fusion protein in some clones) using the Bac-to-Bac® Baculovirus Expression System available from Invitrogen. For more information on the Bac-to-Bac® Baculovirus Expression System, visit www.invitrogen.com.

The LR reaction mix obtained after performing the LR reaction is transformed into competent DH10Bac™ *E. coli* to generate a recombinant bacmid. The high molecular weight recombinant bacmid DNA is isolated and transfected into Sf9 insect cells to generate a recombinant baculovirus that is used for preliminary expression experiments. After the baculoviral stock is amplified, the high-titer stock is used to infect Sf9 insect cells for expression of the recombinant protein of interest.

The expressed proteins are purified by affinity chromatography under high-throughput conditions optimized to obtain maximal protein integrity, function, and activity. Following purification, each protein is assayed for purity and expected molecular weight.

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ProtoArray® Human Protein Microarray, continued

Controls

Various proteins and controls are printed on each ProtoArray® Human Protein Microarray to allow you to verify background and detection conditions during probing. For details, see page 9.

Printing the Human Protein ProtoArray®

The purified human proteins are printed on nitrocellulose-coated slides in a dust-free, temperature, and humidity controlled environment to maintain consistent quality of the microarrays. The arrays are printed using an automated process on an arrayer that is extensively calibrated and tested for printing ProtoArray® Human Protein Microarrays. The management system governing the manufacture of ProtoArray® Human Protein Microarrays is certified to ISO 9001:2000.

Maintaining Stringent Quality Control

The ProtoArray® Human Protein Microarrays are produced using rigorous production and quality control procedures with an integrated data management system to ensure consistent results with every array and maximize inter- and intra-lot reproducibility.

Pre-Printing Quality Control

Prior to production, the arrayer and supporting components are tested and adjusted to production specifications. The quality and performance of pins is critical and all pins are extensively tested and calibrated. To maintain protein stability and function, arrays are printed at 6ºC under controlled environmental conditions.

Post-Printing Quality Control

After production each microarray is visually inspected for obvious defects that could interfere with the experimental results. To control for the quality of the printing process, several microarrays from each lot are probed with an anti-GST antibody. Since the proteins contain a GST fusion tag, probing the microarrays with an anti-GST antibody allows identification of irregular spot morphology or missing spots. The arrays are functionally qualified by probing with Array Control Protein (biotinylated calmodulin kinase) to confirm binding to calmodulin.

Detecting Reciprocal Interactions

ProtoArray® Human Protein Microarrays are ideal for detecting reciprocal interactions since the microarrays are manufactured under highly controlled conditions to ensure maximum protein function.

Once you have identified a positive interaction using the ProtoArray® Human Protein Microarray, use the identified interacting protein from the array as a probe to probe another ProtoArray® Human Protein Microarray to confirm the reciprocal interaction.

For example, perform an initial probing with calmodulin as a probe with a ProtoArray® Human Protein Microarray to detect the interacting protein, calmodulin kinase. Then perform the reciprocal interaction with another human microarray using calmodulin kinase as the probe to detect the interacting protein, calmodulin. The ability to observe reciprocal interactions indicates that the proteins maintain a proper folded state on the array.

ProtoArray® Control Protein Microarray

Introduction

The ProtoArray® Control Protein Microarray contains protein interactors and various controls printed on a nitrocellulose-coated glass slide. The Control Protein Microarrays allow you to validate probing procedures prior to probing the ProtoArray® Human Protein Microarray.

Details about the ProtoArray® Control Protein Microarray are described in this section.

Control Microarray Specifications

The specifications for the ProtoArray® Control Protein Microarray v4.1 are listed below.

Dimensions:	1 inch x 3 inch (25 mm x 75 mm)
Material:	Glass slide coated with a thin layer of nitrocellulose

The nitrocellulose-coated slide is from GenTel® BioSciences, Inc., and is specifically selected for superior performance of the array in the KSI application. APiX™ slides are manufactured by GenTel® BioSciences, Inc. APiX™ technology is covered by US Patent #6,861,251. APiX™ and Gentel® are registered trademarks of GenTel® Biosciences, Inc.

Each microarray has a barcode for tracking samples. The barcode number is also used to retrieve array specific information from the ProtoArray® Central portal (see page 29).

Control Array Specifications

The ProtoArray® Control Protein Microarray specifications are listed below.

The proteins on the microarray are printed in 48 subarrays and are equally spaced in vertical and horizontal directions.

For details on the subarray layout and control spots on the ProtoArray® Control Protein Microarray, go to the ProtoArray® Central Portal at www.invitrogen.com/protoarray.

Total Subarrays:	48 (4 columns x 12 rows)
Subarray Size:	4400 µm x 4400 µm
Subarray Dimensions:	**20 rows x 22 columns**
Median Spot Diameter:	~110 µm
Spot Center to Center Spacing:	200 µm
Distance Between Subarrays:	100 µm
Replicates per Sample:	2

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ProtoArray® Control Protein Microarray, continued

Control Proteins

Various proteins and controls are printed on each ProtoArray® Human Protein and Control Protein Microarray to allow you to verify reagents, background, and detection conditions used during probing. The table below lists the controls printed on each ProtoArray® Microarray.

Protein	Function
Control Spots required for PPI Data Analysis	
Alexa Fluor® Antibody (Rabbit anti-mouse IgG Antibody labeled with Alexa Fluor® 647, Alexa Fluor® 555, and Alexa Fluor® 488)	Serves as a positive control for fluorescence scanning and for orientation of the microarray image.
Bovine Serum Albumin (BSA)	A negative control for non-specific protein interactions.
Biotinylated Anti-mouse Antibody	A positive control for interaction with streptavidin-labeled detection reagent.
Anti-biotin Antibody	Detects biotinylated probes.
V5 Control Protein (biotinylated, V5-tagged control protein)	A positive control for detection with the Anti-V5-Alexa Fluor® 647 Antibody and the streptavidin-labeled detection reagent.
Human IgG Protein Gradient	A positive control for the immune response serum profiling application. Interacts with Alexa Fluor® 647 goat anti-human IgG.
Anti-Human IgG Antibody Gradient (goat anti-human IgG)	A positive control for the immune response serum profiling application. Interacts with serum IgG antibodies which are then bound by Alexa Fluor® 647 goat anti-human IgG.
Yeast calmodulin (Cmd1p)	A positive control for protein-protein interaction application and interacts with the Array Control Protein.
GST Protein Gradient	Serves as a negative control and signals are used by ProtoArray® Prospector software for background and statistical significance calculations.

ProtoArray® Control Protein Microarray, continued

Protein	Function
Control Spots NOT required for PPI Data Analysis	
Alignment Control Kinases	Kinases autophosphorylate and produce signals which are used for orientation of the microarray image; also serves as a positive control for the radiolabel and assay conditions.
Anti-Human IgA Antibody Gradient (goat anti-human IgA)	A positive control for the immune response serum profiling application. Interacts with serum IgA antibodies which are then bound by Alexa Fluor® 647 anti-human IgA.
Control Kinase Substrate	A substrate for the Control Kinase used to verify assay conditions. The Control Kinase phosphorylates the Control Kinase Substrate.
CAMK2A (Calcium/calmodulin-dependent protein kinase II alpha)	A human protein kinase that is used as a positive control for the small molecule profiling application.
Estrogen Receptor Alpha	Binds to tritiated estradiol to produce marker signals which are used for orientation of the microarray image for the radiometric small molecule profiling application.
Human IgA Protein Gradient	A positive control for immune response serum profiling of IgA antibodies. Interacts with Alexa Fluor® 647 anti-human IgA
Mdm2	Serves as a control substrate for ubiquitin ligase profiling.
RanBP2ΔFG	Serves as a control substrate for sumo ligase profiling.

Note

The yeast calmodulin protein (Cmd1p; expressed as described on page 16) is printed on each microarray. When probing the ProtoArray® Control Protein Microarray with the Array Control Protein (*i.e.* biotinylated, yeast calmodulin kinase), these proteins interact. This interaction can be used to verify the reagents and procedures used to probe the human protein microarrays.

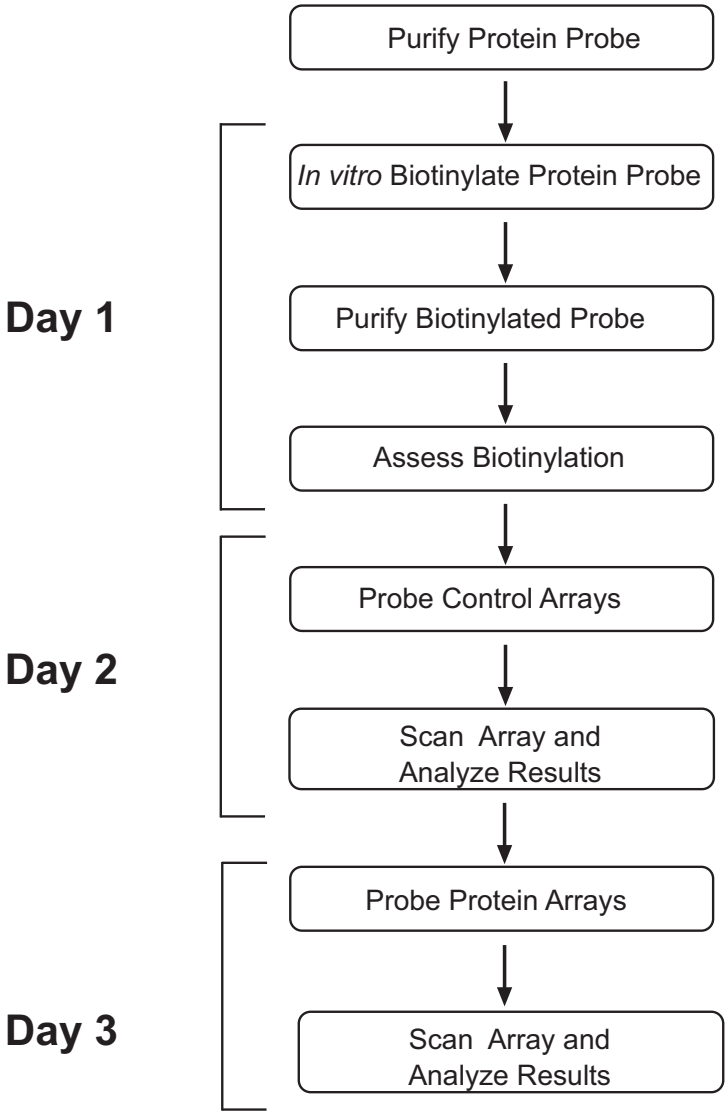
Maintaining Stringent Quality Control

The ProtoArray® Control Protein Microarrays are produced using the same rigorous production and pre-printing and post-printing quality control procedures used to produce the ProtoArray® Human Protein Microarray (page 7). In addition, the control arrays are functionally qualified by probing the arrays with the Array Control Protein (biotinylated, yeast calmodulin kinase) to detect the appropriate interaction with calmodulin.

Experimental Overview

Experimental Timeline

The recommended experimental timeline is outlined below. A detailed experimental workflow is shown on the next page.



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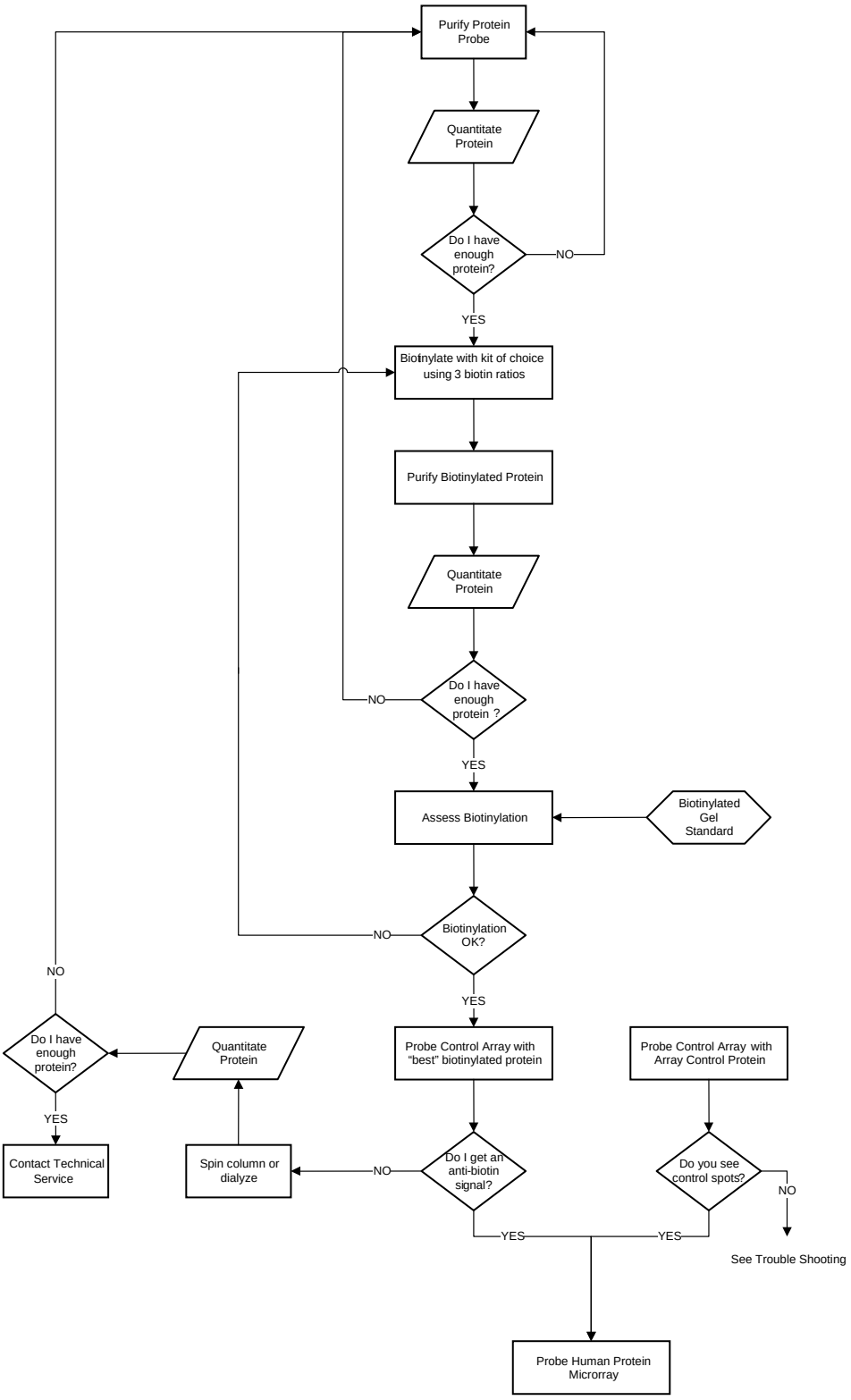
Experimental Overview, continued

Workflow

The experimental workflow for probing the ProtoArray® Human Protein Microarray with your *in vitro* biotinylated probe is shown below.



Methods

Preparing the Protein Probe

Introduction

Before probing the ProtoArray® Microarray, you will need to biotinylate your purified protein of interest and assess the level of biotinylation.

The ProtoArray® Human Protein Microarray PPI Kit *for biotinylated proteins* **does not** come with a module to perform biotinylation of your protein of interest. We recommend using the Biotin-XX Microscale Protein Labeling Kit and FluoReporter® Biotin Quantitation Assay Kit (Invitrogen, Cat. no. B30756) for *in vitro* biotinylation of your protein. Refer to the manual supplied with the kit for detailed instructions.

See below for amount and quality of protein required for probing an array.

Protein Amount and Quality

After you have expressed your protein of interest, follow the guidelines below to purify and prepare the protein probe. You need at least 150 µg of purified protein at a concentration of 2.5 mg/ml.

- Purify the protein probe to > 90% purity as determined by Coomassie® staining.
- Resuspend the purified protein probe in a buffer (≤ 50 mM) that does not contain primary amines (*e.g.* ammonium ions, Tris, glutathione, imidazole, or glycine). If the buffer contains primary amines, sufficiently dialyze proteins against 50 mM HEPES buffer, pH 7.4 with 100 mM NaCl, or PBS.
- Know the approximate molecular weight of your protein.
  Note: The protein must be >15 kDa.
- For proteins purified using metal chelating column chromatography (ProBond™ resin or Ni-NTA resin), perform dialysis against 2 changes of PBS to significantly lower the imidazole concentration.
- If you are using a recombinant protein probe, you may check the functionality of the protein using a method of choice.
- Low concentrations (< 0.1%) of sodium azide or thimerosal in the protein solution have no effect on the biotinylation reaction.

Biotinylation of the Protein Probe

We recommend biotinylating your protein probe at 3 molar ratios of 3:1, 9:1, and 27:1 biotin:protein probe in the final biotinylation reaction mixture.

Biotinylating the protein probes at these molar ratios typically incorporates the following number of biotin molecules per protein:

Molar Ratio	Biotin Molecules/Protein
3:1	1-2
9:1	3-5
27:1	10-15

A 9:1 molar ratio results in a biotinylation efficiency of ~3-5 biotin molecules per polypeptide for average proteins. Proteins with few accessible lysine residues may label poorly with 9:1 molar ratio and may require a 27:1 molar ratio for better biotinylation. Proteins with more lysine residues may over-biotinylate with 9:1 molar ratio and produce better probe quality with a 3:1 molar ratio.

Probing the ProtoArray® Control Protein Microarrays

Introduction

The ProtoArray® Control Protein Microarray allows you to verify probing conditions. Probe the ProtoArray® Control Protein Microarray **prior to** probing the ProtoArray® Human Protein Microarrays.

Instructions are provided in this section to probe the ProtoArray® Control Protein Microarrays supplied with the kit.

ProtoArray® PPI Buffer Modules

The ProtoArray® PPI Buffers Module A and B supplied with the kit include qualified reagents for blocking, washing, and detection during the microarray probing procedure. The pre-made buffers provide consistent results and eliminate the time required to prepare reagents.

ProtoArray® PPI Buffer Module B also includes LifterSlip™ coverslips that hold a small reagent volume to minimize the amount of valuable probe used and prevent evaporation of reagents. Incubation Trays are also included in the module for blocking and washing the microarrays.

Materials Needed

- 2 ProtoArray® Control Protein Microarrays v4.1 (included in the kit and available separately)
- ProtoArray® PPI Buffer Modules A and B (included with the kit)
- Streptavidin-Alexa Fluor® 647 Conjugate (included with the kit; keep on ice in the dark until immediately before use)
- Biotinylated Protein Probe in Casein Washing Buffer (page 16)
- Array Control Protein in Casein Washing Buffer (included in the kit; page 16)
- 2 sterile 50 ml conical tubes
- Shaker (capable of circular shaking at 50 rpm; place the shaker at 4°C)
- Incubation Tray incubation tray, chilled on ice (included with the kit)
- LifterSlip™ (included with the kit)
- Ice bucket
- Deionized water
- *Optional*: Microarray slide holder and centrifuge equipped with a plate holder

Important

Each ProtoArray® Control Protein Microarray can only be used once. Do not re-use the microarray or reprobe the same microarray with another probe.

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Probing the ProtoArray® Control Protein Microarrays, continued

Experimental Outline

1. Block the ProtoArray® Control Protein Microarrays.
2. Probe one array with the biotinylated protein probe and the other with the Array Control Protein.
3. Perform detection with the Streptavidin-Alexa Fluor® 647 Conjugate.
4. Dry the arrays for scanning.

Important Guidelines

Since proteins are sensitive to various environmental factors, each array is produced in an environment-controlled facility to ensure protein integrity and maintain consistency. To obtain the best results and avoid any damage to the array or array proteins, always handle the ProtoArray® Microarrays with care using the following guidelines:

- 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.
- To prevent condensation on the array that may reduce protein activity or alter spot morphology, allow the mailer containing the array to equilibrate at 4°C for at least 15 minutes prior to removing the array from the mailer. Use Blocking Buffer equilibrated at 4°C to immerse the array immediately.
- Perform array experiments at a clean location to avoid dust or contamination. Filter solutions if needed (particles invisible to eyes can produce high background signals and cause irregular spot morphology).
- Avoid drying of the array during the experiment. Ensure the array is completely covered with the appropriate reagent during all steps of the protocol.
- Always dry the array prior to scanning. Scan the array on the same day at the end of the experiment.
- Do not dry the array using compressed air or commercial aerosol sprays.
- Avoid exposing the array to light after probing with Streptavidin-Alexa Fluor® 647 conjugate.

Probes for Control Arrays

Use the following biotinylated proteins to probe the ProtoArray® Control Protein Microarrays:

- **Biotinylated Protein** (provided by user): An interaction of the biotinylated protein with the anti-biotin antibody indicates that the protein is in fact biotinylated, the biotins are accessible in solution, and that the amount of free biotin remaining in the sample is low. Use the biotinylated protein sample that gives the best signal on a Western blot at the lowest biotinylation molar ratio to probe the control array.
- **Array Control Protein:** Reacts with calmodulin printed on the Control Arrays; use to verify probing procedure and reagents.

Continued on next page

Probing the ProtoArray® Control Protein Microarrays, continued

Array Control Protein

The Array Control Protein (included in the kit) is yeast calmodulin kinase (Cmk1p) containing an N-terminal BioEase™ and V5 tag. The presence of the BioEase™ tag facilitates *in vivo* biotinylation of the protein during expression (see www.invitrogen.com for more information about BioEase™ vectors). When probed against the ProtoArray® Control Protein Microarray, the biotinylated calmodulin kinase interacts with calmodulin (Cmd1p) printed on the array. Detecting an interaction between the Array Control Protein and calmodulin indicates that the probing procedure has been performed correctly.

Preparing Buffers

Casein Blocking Buffer

5 ml of Casein Blocking Buffer is needed for each array. Prepare 10 ml of buffer for two arrays as follows:

1. Chill 10 ml buffer to 4°C and add 10 µl of 1 M DTT.
2. Mix well (do not vortex) and store on ice until use.

Casein Washing Buffer

The Casein Washing Buffer supplied in the kit is ready to use. 60 ml of Casein Washing Buffer is needed for each array. Chill buffer to 4°C prior to use.

Preparing the Probes

Array Control Protein (biotinylated calmodulin kinase)

Mix 12 µl of the Array Control Protein (included in the kit) with Casein Washing Buffer to a final volume of 120 µl. Mix well (do not vortex) and store on ice until use.

Biotinylated Protein Probe

You need 120 µl of the protein probe. Use the biotinylated protein sample that gives the best signal on Western blot at the lowest biotinylation molar ratio and dilute the probe to 50 µg/ml in Casein Washing Buffer. Mix well (do not vortex) and store on ice until use.

Before Starting

- Before starting the probing procedure, make sure you have all items on hand especially buffers (above), probes in Casein Washing Buffer (above), Incubation Trays (included in the kit), and LifterSlips™ (included in the kit).
- Make sure the buffers are cold. Store buffers on ice until use. Place the Incubation Trays on ice to chill the tray prior to use.
- Review **Important Guidelines** on page 15 prior to starting the probing procedure.

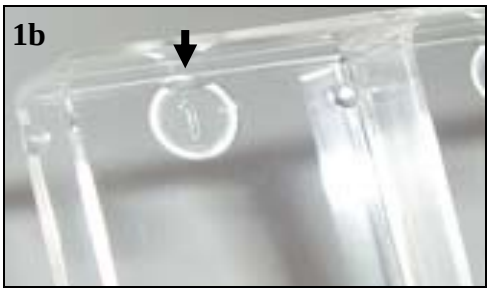
Continued on next page

Blocking Step

Instructions for blocking the control microarray are described below:

1. Remove the mailer containing the ProtoArray® Control Protein Microarray from storage at –20°C and place immediately at 4°C (be sure to use the microarray **before** the expiration date printed on the box).
2. Allow the array to equilibrate in the mailer at 4°C for at least 15 minutes prior to blocking. Failure to do so may result in condensation on the array which can reduce protein activity or alter spot morphology.
3. Place one microarray with the barcode facing up into each well of a chilled Incubation Tray 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).



4. Using a sterile pipette, add 5 ml Casein Blocking Buffer (page 16) equilibrated to 4°C into each chamber with an array. **Avoid pipetting buffer directly onto the array surface.** Gently rock the tray to ensure each array is completely immersed in Casein Blocking Buffer.
5. Incubate the tray for 1 hour at 4°C on a shaker set at 50 rpm (circular shaking).

Continued on next page

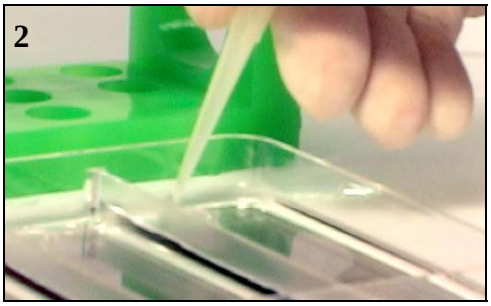
Probing the ProtoArray® Control Protein Microarray, continued

Blocking Step, continued

Protocol continued from the previous page.

6. After incubation, aspirate Casein Blocking Buffer by vacuum or with a pipette. Position the tip of the aspirator or pipette into the indentation at the end of the tray (see figure 1b, previous page) 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.



7. Add 5 ml Casein Washing Buffer and incubate on shaker at 4°C for 5 minutes.
8. Aspirate Casein Washing Buffer (see Step 6).
9. Proceed immediately to **Probing Control Array**.

Continued on next page

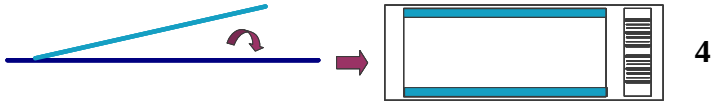
Probing the ProtoArray® Control Protein Microarray, continued

Probing Control Array

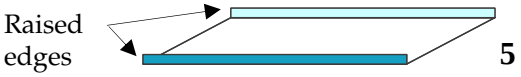
1. Remove array from the 4-well tray by inserting the tip of forceps into the indentation at the numbered end of the tray and gently prying the array upward (see figure 3). Pick up array with a gloved hand taking care to only touch the array by its edges. Gently dry the back and sides of the array on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

2. Pipet 120 µl of the biotinylated protein probe (50 µg/ml) in Casein Washing Buffer (page 16) on top of array.
3. Carefully lower a LifterSlip™ coverslip over the printed area of the array using forceps, as shown below (figure 4).



   The **raised edges of the LifterSlip™ should face the surface of the array** (shown inverted on figure 5 below). If air bubbles are observed under the LifterSlip™ gently raise the LifterSlip™ and slowly lower it again.



4. Incubate for 90 minutes at 4°C keeping the 4-well tray flat with the array facing up (no shaking).
5. Add 5 ml cold Casein Washing Buffer, and remove the LifterSlip™ with forceps, taking care not to scratch the array surface with the LifterSlip™ or forceps. Wash 5 minute with gentle agitation. Remove Casein Washing Buffer by aspiration (see Step 5 of **Blocking Step** for details).
6. Repeat wash steps four more times.

Continued on next page

Probing the ProtoArray® Control Protein Microarrays, continued

Probing Control Array

7. Add 5 ml Alexa Fluor® 647 conjugated streptavidin (1 µg/ml in Casein Washing Buffer) .

   Note: Add the conjugate solution at the indented numeral end of the 4-well tray and allow the liquid to flow across the array surface. To prevent local variations in fluorescence intensity and background, **avoid direct contact with the array** and if at all possible, avoid applying the antibody solution directly on to the array.
8. Incubate for 90 minutes at 4°C with gentle circular shaking (~50 rpms) in the dark.
9. Remove conjugate solution by aspiration (see **Blocking Step**).
10. Wash with 5ml cold Casein Washing Buffer for 5 minutes with gentle agitation. Remove Casein Washing Buffer by aspiration (see **Blocking Step**).
11. Repeat wash step four more times.
12. Remove the array from the 4-well tray using forceps (see Step 1, above).
13. Proceed to **Drying the Arrays**.

Drying the Arrays

1. Insert array into a slide holder and quickly rinse by submerging into a large beaker filled with deionized water. 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® Control Protein Microarray 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.
4. To obtain the best results, scan the array within 24 hours of probing using a fluorescence microarray scanner (see page 27 for details).

Continued on next page

Probing the ProtoArray® Control Protein Microarrays, continued

Data Analysis

After scanning and saving an image of each array, analyze results to identify positive interactors. For more details, see page 28.

1. To acquire data from the scanned image, use the barcode on the array to download the .GAL file from ProtoArray® Central as described on page 29.
2. Use the .GAL file and suitable microarray data acquisition software to acquire pixel intensity values for all features on the control array.
3. Analyze data using the guidelines on page 30 to determine significant signals with the Array Control Protein and your biotinylated protein probe.

   Note: An example of the expected results obtained after probing the Control Arrays is shown on page 32. For **Troubleshooting**, see page 35.
4. After confirming the appropriate interactions on the Control Arrays, proceed to **Probing the ProtoArray® Human Microarray**, next page.

Cleaning the Chamber

At the end of probing experiments, clean the Incubation Tray properly and rinse with sterile water before re-using the Incubation Tray.

Probing the ProtoArray® Human Microarray

Introduction

After using the ProtoArray® Control Protein Microarray to verify the quality of your *in vitro* biotinylated protein probe and the probing conditions, you may proceed to probe the ProtoArray® Human Protein Microarray using your protein probe. Follow the guidelines provided in this section.

Materials Needed

- ProtoArray® Human Protein Microarray (included in the kit)
- ProtoArray® PPI Buffers Module A and B (included in the kit)
- Your biotinylated protein probe in Casein Washing Buffer (page 24)
- Streptavidin-Alexa Fluor® 647 conjugate (keep on ice in the dark until immediately before use)
- Sterile 50 ml conical tube
- Shaker (capable of circular shaking at 50 rpm; place the shaker at 4°C)
- Incubation Tray incubation tray, chilled on ice (included with the kit)
- LifterSlip™ (included with the kit)
- Ice bucket
- Deionized water
- *Optional*: Microarray slide holder and centrifuge equipped with a plate holder

Important

Each ProtoArray® Human Protein Microarray can only be used once. Do not re-use the array or reprobe the same array with another probe.

Experimental Outline

1. Block the ProtoArray® Human Protein Microarray.
2. Probe with your biotinylated protein probe.
3. Detect with Streptavidin-Alexa Fluor® 647 Conjugate.
4. Dry the array for scanning.

Important Guidelines

Follow the **Important Guidelines** on page 15 to obtain the best results with the arrays.

Continued on next page

Probes for Human Protein Arrays

The ProtoArray® Human Protein Microarray PPI Kit *for biotinylated proteins* contains 2 human arrays and can be probed using the different probing options described below. Choose the option that best fits your needs. The recommended protein probe concentration to use for probing the arrays is 5-50 µg/ml.

Probing Options

- You can probe both arrays simultaneously, probing one array with your biotinylated protein probe and the second array with no protein probe (negative control). The negative control allows you to determine which signals are specific to your probe.

  OR

- You can probe one array with your protein probe of interest and the second array with the Array Control Protein supplied with the kit (positive control). The results from the positive control help you to determine signals specific to your probe.

  OR

- You can probe one array with an initial probe concentration or biotinylation molar ratio. If the initial signal is strong with low background, confirm the initial results with the second array using the same experimental conditions. If the initial results indicate weak signal and unacceptable signal-to-noise ratio, probe the second array with a different probe concentration or molar ratio as described in the table below:

Probe first array….	And….	Then Probe Second Array….
With 5 µg/ml probe	Weak signal	With 50 µg/ml probe
With 50 µg/ml probe	High background	With 5 µg/ml probe
With 9:1 molar ratio	Weak signal	With 27:1 molar ratio
With 27:1 molar ratio	High background	With 3:1 or 9:1 molar ratio

Note: To identify protein-protein interactions specific to your protein probe, we recommend probing a second array with another V5-tagged protein probe or the Array Control Protein (see above). Probing using options 1 or 2 allows you to determine the probe-specific interactions and helps you identify non-specific interactions.

Continued on next page

Probing the ProtoArray® Human Microarray, continued

Preparing Buffers

Prepare Casein Blocking Buffer and Casein Washing Buffer as described on page 16.

Preparing Probes

You need 120 µl of your biotinylated protein probe. Use the biotinylated probe that gives the best signal on the Western blot at the lowest biotinylation molar ratio. Dilute the probe to 5-50 µg/ml in Probing Buffer. Mix well (do not vortex) and store on ice until use.

Before Starting

- Before starting the probing procedure, make sure you have all items on hand especially buffers (above), probes in Casein Washing Buffer (above), Incubation Trays (included in the kit), and LifterSlips™ (included in the kit).
- Make sure the buffers are cold. Store buffers on ice until use. Place the Incubation Tray on ice to chill the tray prior to use.
- Review **Important Guidelines** on page 15 prior to starting the probing procedure.

Probing Arrays

The options for probing the array are described on the previous page. Choose the option that best fits your needs.

1. Block microarrays using the procedure described on page 17.
2. Probe the ProtoArray® Human Protein Microarray using the procedure described on page 22.
3. Dry the array as described on page 20.
4. Scan the arrays as described on the next page and analyze results (page 28).

Examples of expected results obtained after probing the ProtoArray® Human Protein Microarray are shown on pages 33.

If you obtain weak signals or high background, see **Troubleshooting**, page 35.

Scanning Arrays

Introduction

Once you have probed the ProtoArray® Microarray with your biotinylated protein, scan the microarray using a fluorescence microarray scanner.

Materials Needed

A suitable fluorescence microarray scanner is needed to scan the ProtoArray® Microarray. A list of scanners that can be used with ProtoArray® Microarrays can be found on the next page. The scanner specifications are listed below.

To acquire ProtoArray® data from the image, the appropriate microarray data acquisition software is needed. The recommended microarray data acquisition software for analysis is GenePix® Pro v6 or later (Molecular Devices Corporation) or ScanArray® Acquisition Software (PerkinElmer, Inc.).

Experimental Outline

1. Insert array into the fluorescence microarray scanner.
2. Adjust scanner settings.
3. Preview the microarray and adjust settings, if needed.
4. Scan the microarray.
5. Align grid over spots and use image analysis software to align features.
6. Export and analyze results.

Scanner Specifications

The fluorescence microarray scanner specifications required to image the ProtoArray® Human Protein Microarray are listed in the table below.

Array Compatibility	**Size**	Standard 1" x 3" or 25 mm x 75 mm microscope slides
	Thickness	1 mm
Detection	**Light and Detector Orientation**	Facing array
	Scanned Area	22 mm x 73 mm
	Focus	Auto focus or adjustable (± 200 µm)
	Excitation	635 nm or equivalent
	Detection limit	0.1 fluor/µm^2
	Resolution	≤10 µm
	Dynamic Range	>3 orders of magnitude
	Output	16-bit TIFF

Scanning Arrays, continued

Recommended Scanners

The following scanners are **compatible** for scanning ProtoArray® Human Protein Microarray:

- GenePix® 4000A (Molecular Devices Corporation)
- GenePix® 4000B (Molecular Devices Corporation)
- GenePix® Professional 4200A (Molecular Devices Corporation)
- GenePix® Personal 4100A (Molecular Devices Corporation)
- ScanArray® Lite (PerkinElmer, Inc.)
- ScanArray® Express (PerkinElmer, Inc.)
- ScanArray® Express HT (PerkinElmer, Inc.)
- LS Series Laser Scanner (Tecan Group AG)

The following scanners **may be compatible** with ProtoArray® Human Protein Microarray:

- AlphaArray® Reader (Alpha Innotech Corporation)
- arrayWoRx®e 4-Color Biochip Reader (Applied Precision, LLC)
- SpotLight™ (TeleChem International, Inc.)

The following scanners are **not compatible** with ProtoArray® Human Microarray:

- GeneChip® Scanner 3000 (Affymetrix, Inc.)
- DNA Microarray Scanner (Agilent Technologies, Inc.)

Note

Unlike most DNA microarrays, you will scan the ProtoArray® Human Protein Microarray using only one color.

Continued on next page

Scanning Arrays, continued

Scanning Procedure

A brief procedure for scanning the ProtoArray® Human Protein Microarray with a fluorescence microarray scanner at 635 nm is described below.

For details on using a specific scanner, refer to the manual supplied with the scanner.

The scanning time for each array is ~7–8 minutes.

1. Start the appropriate array acquisition and analysis software on the computer connected to the fluorescence microarray scanner.
2. Open the microarray enclosure on the scanner.
3. Place the ProtoArray® Human Protein Microarray in the holder such that the nitrocellulose-coated side of the array faces the laser source and the barcode on the array is closest to the outside of the instrument.
4. Close the microarray enclosure on the scanner.
5. Set the following settings to image the microarray:
   - Wavelength: 635 nm
   - PMT Gain: 600
   - Laser Power: 100%
   - Pixel Size: 10 µm
   - Lines to Average: 1.0
   - Focus Position: 0 µm
6. Perform a preview to quickly scan the microarray. Adjust the PMT Gain, if needed.

   Note: The image should have very few saturated spots (white). Adjust settings such that the Alexa Fluor® Ab spots are at or near the pixel saturation.
7. Select the area of the array to scan in detail (include the barcode in the scan area for maintaining experimental records) and then scan the array to provide a high-resolution image.
8. After acquiring the image, save the image to a suitable location as "multi-image TIFF" file. Be sure the barcode number is included in the name of the image.

   Note: Examples of expected image scans of control and human arrays are shown on pages 32-33.
9. Open the microarray enclosure and remove the microarray from the holder.
10. Proceed to download lot specific information available on the ProtoArray® Central Portal, next page.

Note

To orient the results obtained from the .GAL file and ProtoArray® Prospector with the array image, position the microarray image such that the barcode is at the bottom of the image. In this orientation, the top left corner of the microarray image is Block 1.

Data Acquisition and Analysis

Introduction

After scanning and saving an image of the array, download the protein array lot specific information (including the .GAL file) from the ProtoArray® Central Portal. Use the lot specific information to acquire and analyze the data to identify protein-protein interactions.

Note: To familiarize yourself with the array and subarray layout, you may also download a file showing the subarray layout from ProtoArray® Central. To access the file, go to www.invitrogen.com/protoarray and click on the **ProtoArray® Lot Specific Information** link under **BioMarker Discovery Resources**.

Important

While downloading the lot specific information files, ensure that you are downloading files that are associated with your specific barcode on the array. Since lot specific information files are updated frequently based on recently available sequence or protein information, make sure that you download the latest version of the lot specific information files.

GAL File

The .GAL (GenePix Array List) files describe the location and identity of all spots on the Human and Control microarrays and are used with the microarray data acquisition software to generate files that contain pixel intensity information for feature/spot and non-features of the array.

The .GAL files are available for downloading from the ProtoArray® Lot Specific Information available on ProtoArray® Central, see below.

Note: The .GAL files are text files that contain the data in a format specified by GenePix® Pro Microarray data acquisition software. If you are using any other microarray data acquisition software, you can use data from the .GAL files to generate files that are compatible with your microarray data acquisition software.

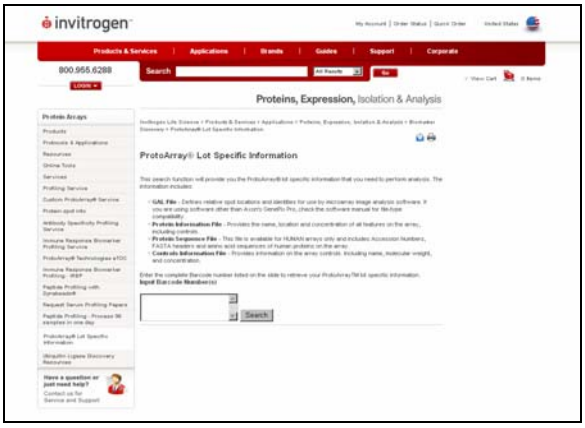
Continued on next page

ProtoArray® Central

The ProtoArray® Central Portal provides a web-based user interface to retrieve ProtoArray® Lot Specific Information. This information (.GAL file) is required for acquiring the array data.

If the scanner computer is connected to the Internet, connect to the portal. If the scanner computer is not connected to the internet, download the array-specific information to portable media as described below and then download the information onto the scanner computer.

1. Connect to the portal at www.invitrogen.com/protoarray and then click on the **ProtoArray® Lot Specific Information** link under **BioMarker Discovery Resources**.
2. The ProtoArray® Lot Specific Information page is displayed.
3. Enter the array barcode in the Input Barcode Number(s) box. Click on the Search button.



4. For each input barcode, the following files are displayed:

   .GAL file (LotNumber.gal):

   This file is essential for data acquisition by the software and defines spot locations and identities of all protein spots on the array. The file also includes the "equivalent solution protein concentration" in nM for use during data analysis.

   Protein Information File (LotNumber_info.txt):

   This file contains a listing and description of human proteins on the array.

   Protein Sequence File (LotNumber_seq.txt):

   This tab-delimited text file lists the GenBank® accession number, Ultimate™ ORF Clone ID number (if available), FASTA header, and amino acid sequence of the ORF for each array protein.

   Control Data File (LotNumber_control.txt):

   This file contains a description of control spots on the array.

   Slide Information File (LotNumber_slide.txt):

   This file contains a listing of all barcodes associated with a specific lot of arrays.

   Note: The file size for some files such as the Protein Sequence File may be larger than 1 MB.

Continued on next page

Data Acquisition and Analysis, continued

Data Acquisition

1. Start the microarray data acquisition software on the computer and open the saved image (.tiff) from Step 8, page 27.
2. To acquire data from ProtoArray® experiments:
   - For GenePix® Pro Software, download the .GAL files from ProtoArray® Central for control or protein arrays, which defines the array grid required by the microarray data acquisition software.
   - For other microarray data acquisition software, use data from the .GAL files from ProtoArray® Central for control or protein arrays to generate files that are compatible with your microarray data acquisition software to define the array grid.

   Scroll through the image to ensure that the grid is in the proper location for each subarray. Adjust the subarray grid, if needed.
3. After the grid is properly adjusted and all features are aligned, save/export the results as a .GPR (GenePix® Results) file for data analysis using ProtoArray® Prospector. The results contain the pixel intensity information for each spot/feature on the array and information on additional parameters depending on the type of software used for data acquisition.

Note: If you wish to perform data analysis using Microsoft® Excel, save/export the results with an .xls extension or rename the .tab or .gpr file using the .xls extension.

Data Analysis Using ProtoArray® Prospector

The ProtoArray® Prospector software quickly analyzes the data acquired from the image acquisition software and easily identifies statistically significant interactors, saving you time and effort. In addition, the software has features that allow you to modify the analysis method and compare data obtained from different arrays.

The ProtoArray® Prospector software and manual are available free-of-charge to ProtoArray® Microarray users. To download the ProtoArray® Prospector software and manual, go to www.invitrogen.com/protoarray, and click **Online Tools** link under **BioMarker Discovery Resources**.

The ProtoArray® Prospector software currently accepts the output files (.GPR) generated by the GenePix® Pro microarray data acquisition software, and analyzes the data using specified algorithms to generate a list of human proteins showing significant interactions with the probe. If .GPR files are not available, consult the ProtoArray® Prospector User Manual for guidelines to format a results file that is compatible for import into ProtoArray® Prospector.

ProtoArray® Prospector Results

After data analysis, ProtoArray® Prospector presents a summary of the analyzed data in a table format (see ProtoArray® Prospector manual for details).

The proteins that score as positive in the experiment are proteins that satisfy the basic program options.

We recommend validating the protein-protein interaction by ProtoArray® Technology or other methods as described on the next page.

Continued on next page

The Next Step

After identifying a positive interaction on the ProtoArray® Human Protein Microarray, you may validate the protein-protein interaction using the ProtoArray® Technology or other methods.

Using the ProtoArray® Technology, validate the protein-protein interactions by performing experiments with additional arrays to ensure:

- **Reproducibility:** Probe the ProtoArray® Human Protein Microarray using a similar or a different probe concentration to observe similar interactions.
- **Specificity:** Probe a ProtoArray® Human Protein Microarray with different biotinylated proteins to identify interactions specific to your protein probe of interest and also identify any non-specific interactions.
- **Reciprocal Interactions:** Determine reciprocal interactions using a purified protein probe (see page 34 for an example).

Other methods for validating protein-protein interactions include:

- Yeast Two-Hybrid Systems (page vi)
- Co-immunoprecipitation
- Gel-shift assay

Accessing Clones

Since the majority of human proteins printed on the array are derived from the Ultimate™ ORF Clone Collection or purified proteins (protein kinases) available from Invitrogen, it is very easy to order the clone or protein corresponding to the protein identified on the array and validate the interaction.

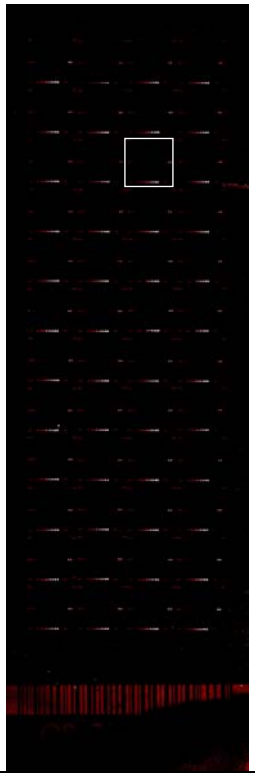
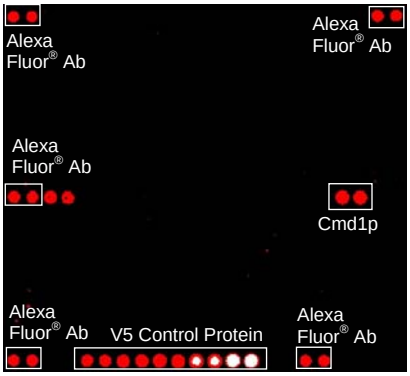
Visit www.invitrogen.com/clones to access our clone collections. Each Ultimate™ ORF Clone is full insert-sequenced and guaranteed to match the corresponding GenBank® amino acid sequence. Contact Technical Support (page 38) to order the purified protein kinases printed on the array or to request information about custom production of additional proteins present on the array.

Expected Results

Control Array Probing Results

Results obtained after probing the ProtoArray® Control Protein Microarray v4.1 with the Array Control Protein (BioEase™-V5-tagged calmodulin kinase) are shown.

Image showing calmodulin (Cmd1p) signal when probed with 5 µg/ml of the Array Control Protein

Array image	Boxed Area shown in detail
	

- Alexa Fluor® Ab signal
  This is an antibody labeled with Alexa Fluor® 647. The fluorescent antibody signals indicate that the array has been properly scanned, and are used as reference spots to orient the microarray and help assign spot identities.
- Cmd1p signal
  The Array Control Protein (V5-tagged calmodulin kinase) binds to the calmodulin printed on the array. The signal is used to verify the probing procedure.
- BioEase™ (biotin) V5 control protein signal
  The Anti-V5-Alexa Fluor® 647 Antibody binds to a control protein (V5 Control) containing an N-terminal V5 tag printed on the microarray. The signals indicate that the antibody is functional and probing is performed properly. The signal is also used to check the background.

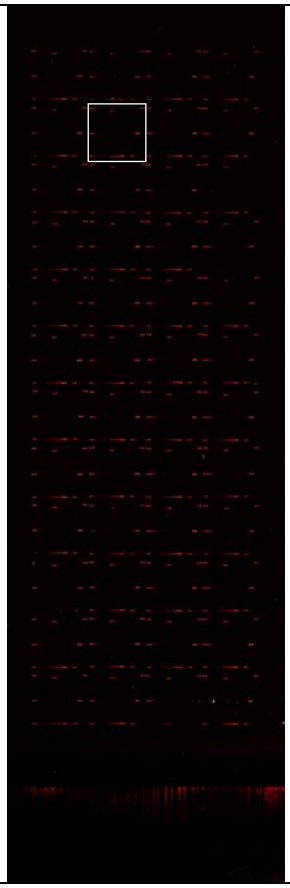
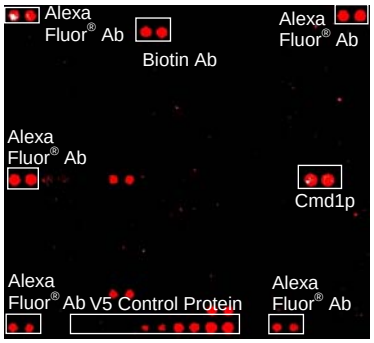
Continued on next page

Expected Results, continued

ProtoArray® Human Protein Microarray v4.1 Probing Results

The results obtained after probing the ProtoArray® Human Protein Microarray v4.1 with 50 µg/ml of the Array Control Protein (*i.e.* BioEase™-V5-tagged biotinylated calmodulin kinase) probe is shown below.

Human Array probed with 50 µg/ml Array Control Protein and Streptavidin-Alexa Fluor® 647 Conjugate

Array Image	Boxed Area shown in detail
	

- Anti-biotin Ab signal
  Biotinylated proteins bind to the Anti-biotin antibody printed on the microarray.

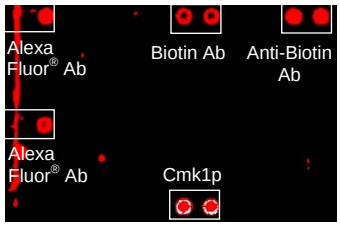
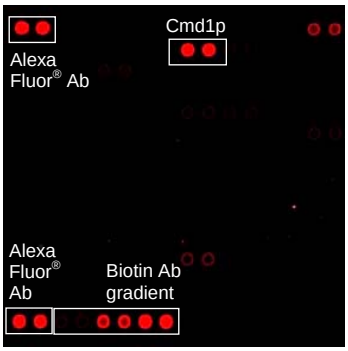
  Note: The Array Control Protein contains an N-terminal BioEase™ and V5 epitope tag. The BioEase™ tag facilitates *in vivo* biotinylation of the protein during expression.
- Biotin Ab signal
  A biotinylated anti-mouse antibody is printed on the microarray. The Streptavidin-Alexa Fluor® 647 conjugate binds to the biotinylated anti-mouse antibody.

Continued on next page

Expected Results, continued

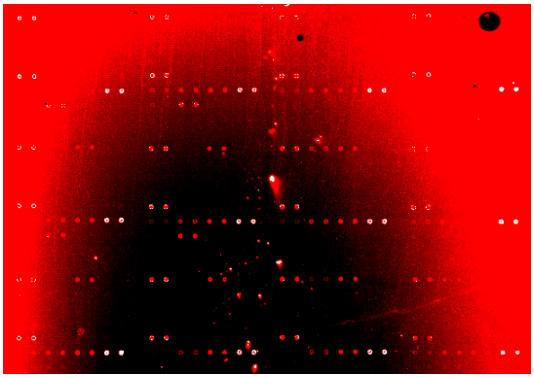
Example of Reciprocal Interaction

Demonstration of reciprocal interactions provides more confidence that the interactions observed most likely result from a direct protein-protein interaction between the labeled protein probe and the array protein. An example of a reciprocal interaction observed after probing the ProtoArray® Yeast Proteome Microarray nc v1.0 is shown below. Reciprocal interactions have been also been demonstrated with the ProtoArray® Human Protein Microarray (results not shown).

Biotinylated Calmodulin (probe) interacting with calmodulin kinase (Cmk1p)	Biotinylated Calmodulin kinase (probe) interacting with calmodulin (Cmd1p)
	

Example Showing High Background

In this example, the ProtoArray® Control Protein Microarray was dried during the probing procedure, producing high background.



Troubleshooting

Introduction

The table below provides some solutions to possible problems you may encounter when using the ProtoArray® Human Protein Microarray PPI Kit.

Problem	Cause	Solution
***In vitro* Biotinylation**		
No signal after Western detection	Poor or incomplete transfer	Monitor the transfer of pre-stained protein standard bands to determine the transfer efficiency.
	Insufficient exposure time	Increase the exposure time.
	Incorrect gel used	Use a suitable percentage gel to separate your protein of interest.
Additional biotinylated bands observed	Protein impurities present that undergo biotinylation	Impurities may cause high background during probing. Purify protein to remove impurities and perform biotinylation to ensure the absence of additional biotinylated bands.
Control Array Results		
No signal	Incorrect scanning or imaging	Be sure to scan the array at 635 nm or equivalent and place the array in the slide holder such that the proteins on the array are facing the laser source. If scanning is performed correctly, the spots corresponding to the Alexa Fluor®-labeled antibody will be visible.
		Use the recommended settings (page 27) to obtain a good image.
Weak or no signal with biotinylated protein probe against the anti-biotin antibody	Presence of free biotin	Purify the protein after biotinylation using the spin column supplied in the kit.
Weak or no signal with biotinylated calmodulin kinase probe	Incorrect probing procedure	Follow the recommended protocol for probing. Be sure all incubations are performed at 4°C. Prepare the Casein Blocking Buffer **fresh** as described on page 16.
		Do not allow the array to dry during the probing procedure.
		Avoid prolonged exposure of the Streptavidin-Alexa Fluor® 647 Conjugate to light.
	Incorrect scanning or imaging	See above.

Continued on next page

Problem	Cause	Solution
Control Array Results, continued		
High background	Improper blocking	Prepare the Casein Blocking Buffer **fresh** as described on page 16.
	Improper washing	For the best results, perform the recommended washing steps.
	Array dried during probing	Do not allow the array to dry during probing.
	Array not dried properly before scanning	Dry the array as described on page 20 before scanning.
Uneven background	Uneven blocking or washing	During the blocking or washing steps, ensure the array is completely immersed in Casein Blocking Buffer or Casein Washing Buffer and use at least 30 ml buffer in the Incubation Tray to cover the array completely with buffer.
	Improper washing	To obtain the best results, perform the recommended washing steps.
	Portions of array have dried	Do not allow the array to dry during probing.
	Improper array handling	Always wear gloves and avoid touching the surface of the array with gloved hands or forceps. Take care while inserting the array into the Incubation Tray to avoid scratching the array surface.
	Biotinylated protein probe not applied properly	Apply the probe solution and LifterSlip™ to the array as described in the manual. To avoid drying of the array, make sure the LifterSlip™ covers the printed area of the array and adjust the LifterSlip™ as needed.
	Probe or detection reagents contain precipitates	Centrifuge the probe or detection reagents to remove precipitates prior to probing the array.

Continued on next page

Problem	Cause	Solution
Human Protein Array Results		
Weak or no signal with biotinylated protein probe	Poor biotinylation of protein probe	Refer to the manual for your biotinylation kit for details. See page 23 for biotinylation guidelines.
	Low probe concentration	Perform probing with higher probe concentration or increase the incubation time. Use the probe biotinylated at a higher molar ratio or perform biotinylation at a higher molar ratio.
	Incorrect scanning or imaging	Scan the array at 635 nm or equivalent and place the array in the slide holder such that the proteins on the array are facing the laser source.
	Interaction conditions too stringent	Decrease the number of washes. Perform probing and washing in the absence of or in lower concentration of detergent or salts.
High background	Improper blocking	Prepare the Casein Blocking Buffer **fresh** as described on page 16.
	Improper washing	To obtain the best results, perform the recommended washing steps.
	Array dried during probing	Do not allow the array to dry during probing.
	Array not dried properly before scanning	Dry the array as described on page 20 before scanning.
	High probe concentration	Decrease the probe concentration to 5 µg/ml or decrease the incubation time.
	Streptavidin-Alexa® Fluor 647 Conjugate cross-reactivity	Probe a human array using only the Streptavidin-Alexa® Fluor 647 Conjugate without the protein probe to detect cross-reactivity with the conjugate only.
Uneven background	See previous page for details	See previous page for details.

Appendix

Technical Support

Web Resources

Visit the Invitrogen website at **www.invitrogen.com** for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
- Complete technical support contact information
- Access to the Invitrogen Online Catalog
- Additional product information and special offers

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For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our Web page (www.invitrogen.com).

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