invitrogen™

ProtoArray® Protein Microarray PPI Kits for Biotinylated Proteins

For detecting protein-protein interactions (PPI) using a human or yeast protein microarray and a biotinylated protein

Catalog nos. PA011, PA0121011, and PAH0524011

Version D
18 October 2006
25-0786

User Manual

Table of Contents

Kit Contents and Storage

Types of Kits

This manual is supplied with the following kits.

Product	Catalog no.
ProtoArray® Human Protein Microarray PPI Complete Kit v4.0 *for biotinylated proteins*	PAH0524011
ProtoArray® Yeast Proteome Microarray PPI Complete Kit v1.1 *for biotinylated proteins*	PA0121011
ProtoArray® Protein-Protein Interaction Application Kit *for biotinylated proteins*	PA011

Kit Components

The ProtoArray® PPI Kits for Biotinylated Proteins include the following components. For a detailed description of the contents of each component, see pages vi-ix.

Note: Catalog nos. PAH0524011 and PA0121011 include **two** ProtoArray® Human Protein or Yeast Proteome Microarrays, as appropriate and **two** ProtoArray® Control Protein Microarrays.

Component	Catalog no.		
	PAH0524011	**PA0121011**	**PA011**
ProtoArray® Human Protein Microarray nc **v4.0**	√		
ProtoArray® Yeast Proteome Microarray nc **v1.1**		√	
ProtoArray® Control Protein Microarray nc **v4.0**	√	√	
Array Control Protein	√	√	
Streptavidin-Alexa Fluor® 647 Conjugate	√	√	√
ProtoArray® PPI Buffer Module A	√	√	√
ProtoArray® PPI Buffer Module B	√	√	√
ProtoArray® Mini-Biotinylation Module	√	√	
ProtoArray® Biotinylation Purification Module	√	√	
ProtoArray® Biotinylation Assessment Module	√	√	

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Shipping and Storage

The components included in the ProtoArray® PPI Kits for Biotinylated Proteins are shipped as detailed below. Upon receipt, store as indicated.

All kit components are stable for 12 months when stored properly.

Components	Shipping	Storage
ProtoArray® Human Protein Microarray nc v4.0	Blue ice	-20°C
ProtoArray® Yeast Proteome Microarray nc v1.1	Blue ice	-20°C
ProtoArray® Control Protein Microarray nc v4.0	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® Mini-Biotinylation Module	Dry ice	-20°C
ProtoArray® Biotinylation Purification Module	Blue ice	4°C
ProtoArray® Biotinylation Assessment Module	Blue ice	4°C

ProtoArray® Human or Yeast Microarrays

Each ProtoArray® Microarray PPI Complete Kit contains mailers with the following ProtoArray® Microarrays:

- Human Kit (Catalog no. PAH0524011): Contains two ProtoArray® Human Protein Microarrays nc v4.0
- Yeast Kit (Catalog no. PA0121011): Contains two ProtoArray® Yeast Proteome Microarrays nc v1.1

Store the microarrays at -20°C.

For details on array specifications, see pages 6-11.

ProtoArray® Control Reagents

Each ProtoArray® Microarray PPI Complete Kit includes the following control reagents. **Store the microarray and Array Control Protein at -20°C.**

Item	Composition	Amount
ProtoArray® Control Protein Microarray nc v4.0	--	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 11. For information about the Array Control Protein, see page 32.

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Streptavidin-Alexa Fluor® 647 Conjugate

The ProtoArray® Microarray PPI Complete Kits and ProtoArray® Protein-Protein Interaction Application Kit each contain 1 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
Bovine Serum Albumin (BSA)	30% BSA in 0.85% NaCl	30 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 4°C.

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

Item	Composition	Amount
ProtoArray® Blocking Buffer (10X)	10X PBS, pH 7.4 1% Tween 20	12 ml
ProtoArray® Probe Buffer (5X)	5X PBS, pH 7.4 0.25% Triton X-100 25% Glycerol	175 ml
$MgCl_2$	1 M $MgCl_2$ in deionized water	4 ml
HybriSlip™ Cover Slip	60 mm x 22 mm, RNase-free	5 cover slips per pack
Array Chambers	--	2

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ProtoArray® Mini-Biotinylation Module

The ProtoArray® Mini-Biotinylation Module includes the following reagents. **Store at -20°C.**

Note: Sufficient reagents are included to perform 4 *in vitro* biotinylation reactions.

Component	Composition	Amount
Biotin-XX sulfosuccinimidyl ester, sodium salt	Lyophilized	100 µg
Sterile water	--	1 ml
Control Protein (BSA)	2.5 µg/µl in PBS, pH 7.4	20 µl
Biotinylation Gel Standard (Biotinylated BSA)	20 pmoles biotin conjugated per ml of BSA, in 1X NuPAGE® LDS Sample Buffer	40 µl
1X NuPAGE® LDS Sample Buffer	Proprietary	1 ml
10X NuPAGE® Reducing Agent	500 mM stabilized dithiothreitol (DTT)	250 µl

ProtoArray® Biotinylation Purification Module

The ProtoArray® Biotinylation Purification Module includes the following reagents. **Store Purification Resin at 4°C and Spin Columns and Collection Tubes at room temperature.**

Note: Sufficient reagents are included to perform 4 purifications.

Component	Composition	Amount
Purification Resin	50% slurry in 50 mM HEPES, pH 7.4 containing 0.1 M NaCl and 1 mM sodium azide	3.6 ml
Spin Columns with Collection Tubes	--	4
Collection Tubes (additional)	--	4

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ProtoArray® Biotinylation Assessment Module

The ProtoArray® Biotinylation Assessment Module includes the following reagents. **Store at 4°C.**

Note: Sufficient reagents are included to perform 2 Western detections.

Component	Composition	Amount
Western Blocking Buffer A	Concentrated buffered saline solution containing detergent	8 ml
Western Blocking Buffer B	Concentrated Hammersten casein solution	8 ml
Western Washing Buffer (16X)	Concentrated buffered saline solution containing detergent	20 ml
Chemiluminescent Substrate	Ready-to-use solution of CDP-Star® chemiluminescent substrate for alkaline phosphatase	5 ml
Chemiluminescent Substrate Enhancer	Nitro-Block-II™ enhancer	250 µl
Streptavidin-Alkaline Phosphatase (AP) Conjugate	Supplied in 3 M NaCl, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, and 30 mM triethanolamine, pH 7.6 The conjugate has 1600-2600 units per ml of alkaline phosphatase activity and a concentration from 0.75-1.2 mg/ml.	25 µl

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 54).

Product	Quantity	Catalog no.
ProtoArray® Human Protein Microarray nc v4.0	1 array	PAH052401
ProtoArray® Yeast Proteome Microarray nc v1.1	1 array	PA012101
ProtoArray® Control Protein Microarray nc v4.0	1 array	PA1007
ProtoArray® Protein-Protein Interaction Buffer Modules	1 kit	PA014
ProtoArray® Human Protein Microarray PPI Complete Kit v4.0 *for V5 epitope-tagged proteins*	1 kit	PAH0524013
ProtoArray® Yeast Proteome Microarray PPI Complete Kit v1.1 *forV5 epitope-tagged proteins*	1 kit	PA0121013
ProtoArray® Protein-Protein Interaction Application Kit *for V5 epitope-tagged proteins*	1 kit	PA013
ProtoArray® Mini-Biotinylation Kit	1 kit	AL-01
ProtoArray® Human Protein Microarray KSI Complete Kit v4.0 *for kinase substrate identification*	1 kit	PAH0524065
ProtoArray® Yeast Proteome Microarray KSI Complete Kit v1.1 *for kinase substrate identification*	1 kit	PA0121065
ProtoArray® Kinase Substrate Identification Application Kit	1 kit	PA015
ProtoArray® Human Protein Microarray mg v4.0	1 array	PAH052406
ProtoArray® Yeast Proteome Microarray mg v1.1	1 array	PA012106
ProtoArray® Control Protein Microarray mg v4.0	1 array	PA1002
ProtoArray® Immune Response Biomarker Profiling Application Kit	1 kit	PA015
ProQuest™ Two-Hybrid System	1 kit	PQ10002-01
ProQuest™ Two-Hybrid System with Gateway® Technology	1 kit	PQ10001-01
Phosphate-Buffered Saline (PBS), 1X	500 ml	10010-023

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 is available from Invitrogen. For details on these products, visit our website at www.invitrogen.com or contact Technical Support (page 54).

Introduction

Overview

Introduction

The ProtoArray® Human Protein and Yeast Proteome Microarray PPI (protein-protein interaction) Kits for Biotinylated Proteins allow rapid and efficient detection of human or yeast protein-protein interactions using a biotinylated protein probe of interest. The ProtoArray® Human Protein Microarray nc contains thousands of purified human proteins, while the ProtoArray® Yeast Proteome Microarray nc contains >4000 purified yeast proteins from *Saccharomyces cerevisiae*. In both cases, the proteins are printed in duplicate on a nitrocellulose (nc)-coated glass slide. See below for an overview of the system.

ProtoArray® Microarray PPI Applications

ProtoArray® Human Protein and Yeast Proteome Microarrays allow you to:

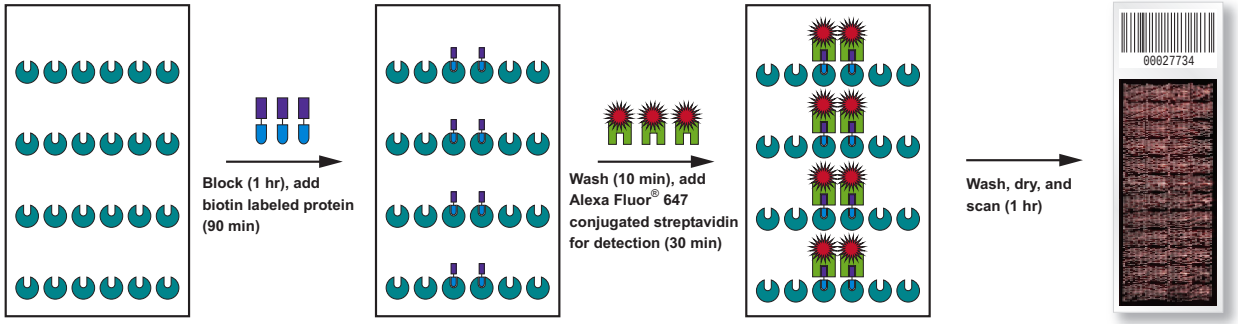
- Detect novel protein-protein interactions
- Validate previously observed protein-protein interactions
- Confirm positive interactions using the identified interacting protein on the array as a probe in reciprocal experiments
- Test various experimental conditions for your protein-protein interactions

System Overview

To use the ProtoArray® Human Protein or Yeast Proteome Microarray PPI Kits, you will:

- *In vitro* biotinylate your protein of interest using the reagents supplied in the kit
- Use the biotinylated protein to probe the ProtoArray® Control Protein Microarray nc to verify protein biotinylation and probing conditions.
- Probe the ProtoArray® Human Protein or Yeast Proteome Microarray nc 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 15.



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Advantages

Using the ProtoArray® Human Protein or Yeast Proteome Microarray PPI Kits 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 reagents for *in vitro* biotinylation, buffers, and detection reagents for probing, eliminating the need to prepare reagents
- Includes controls to verify biotinylation and Western detection protocols
- Allows screening of your protein of interest against thousands of human or yeast 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 and yeast proteins printed on the microarray contain a GST (Glutathione-S-Transferase) fusion tag and some proteins also contain polyhistidine (6x) tag, **do not** use an anti-GST antibody or anti-polyhistidine antibody for detecting interactions on a ProtoArray® Human or Yeast Protein Microarray nc. We strongly recommend that you probe the ProtoArray® Human or Yeast Protein Microarray nc with only your detection reagent to detect signals resulting due to interactions between the detection reagent and proteins printed on the array.
- The ProtoArray® Microarrays are not compatible for use with Alexa Fluor® 555 or Cy3™ dyes. Use of these fluorescent dyes results in high background on the array as the nitrocellulose surface has high intrinsic fluorescence at the wavelength used to visualize Alexa Fluor® 555 or Cy3™ dyes.

Purpose of the Manual

This manual provides the following information:

- An overview of the ProtoArray® Human Protein, Yeast Proteome, and Control Protein Microarrays
- Guidelines and instructions to *in vitro* biotinylate your protein probe
- 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 Kits

The ProtoArray® Human Protein or Yeast Proteome Microarray PPI Complete Kits for Biotinylated Proteins include the following major components:

- The ProtoArray® Human Protein or Yeast Proteome Microarray, a high-density protein microarray that allows you to screen your protein of interest (protein probe) against thousands of human proteins or the *Saccharomyces cerevisiae* proteome, respectively
- The ProtoArray® Control Protein Microarray nc and the Array Control Protein for verification of the probing conditions and background levels
- The ProtoArray® Mini-Biotinylation Module for *in vitro* biotinylation of the protein probe
- The ProtoArray® Biotinylation Purification Module for removing free biotin from your biotinylation reaction
- The ProtoArray® Biotinylation Assessment Module for validation of the level of biotinylation achieved in the protein probe
- 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 and Yeast Proteome Microarrays

The ProtoArray® Human Protein and Yeast Proteome Microarrays are high-density protein microarrays containing human or *S. cerevisiae* proteins, respectively. 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 or *S. cerevisiae* 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 Complete Kit 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 or *S. cerevisiae* proteins within a day to identify protein-protein interactions.

For array specifications and more details on how the human and yeast proteins are prepared, see pages 6-11.

Continued on next page

Description of Kit Components, Continued

ProtoArray® Control Protein Microarray

The ProtoArray® Control Protein Microarray nc contains human and yeast protein interactors and 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 or Yeast Proteome Microarray nc.

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 11.

ProtoArray® Mini-Biotinylation and Purification Module

To detect protein-protein interactions on the ProtoArray® Human Protein or Yeast Proteome Microarray nc, the protein probe must contain a label or tag to visualize the interaction of the probe with array proteins. The extremely high affinity of the biotin-streptavidin interaction makes biotin-protein conjugation an attractive method for probe labeling.

The *ProtoArray® Mini-Biotinylation Module* provides a simple and efficient method to biotinylate small amounts of your protein probe using water-soluble Biotin-XX sulfosuccinimidyl ester. The module includes sufficient reagents to biotinylate your protein probe at 3 molar ratios (page 17). The biotinylated protein probe is detected using streptavidin conjugated to the fluorescent dye, Alexa Fluor® 647 (see next page), providing signal amplification and increased sensitivity.

After *in vitro* biotinylating the protein, the unconjugated or free biotin must be removed from the protein preparation as free biotin interferes with the probing procedure and increases the background on the array. The *ProtoArray® Biotinylation Purification Module* provides spin columns and purification resin to rapidly remove free biotin by gel filtration.

ProtoArray® Biotinylation Assessment Module

Since each protein is different, the number of biotin molecules conjugated to the protein varies. To prevent under-biotinylation of the protein probe resulting in sub-optimal sensitivity or over-biotinylation of the protein probe resulting in loss of protein function, it is important to verify and assess the biotin conjugation reaction.

The *ProtoArray® Biotinylation Assessment Module* allows verification and assessment of the *in vitro* biotinylation reaction using Western detection. The module includes a Biotinylation Gel Standard, buffers, and detection reagents to perform Western transfer and chemiluminescent detection using Streptavidin-Alkaline Phosphatase (AP) conjugate. The band intensity of the protein probe is compared to the Biotinylation Gel Standard to verify biotinylation and assess the level of biotinylation. Based on the Western results, you can choose the protein biotinylated at a suitable level to probe the array and minimize erroneous results due to the use of over- or under-biotinylated probe.

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Description of Kit Components, Continued

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 the ProtoArray® Microarrays. The pre-made buffers provide consistent results and eliminate the time required to prepare reagents.

ProtoArray® PPI Buffers Module B includes HybriSlip™ cover slips that hold a small reagent volume to minimize the amount of valuable probe used and prevent evaporation of reagents. Array Chambers are also included in the module for 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 and Yeast Proteome Microarray PPI Kits 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® PPI Application Kit

The ProtoArray® Protein-Protein Interaction Application Kit includes ProtoArray® PPI Buffer Modules A and B and the Streptavidin-Alexa Fluor® 647 Conjugate **only**. You will need to purchase a ProtoArray® Human Protein, Control Protein, or Yeast Proteome Microarray nc separately from Invitrogen before performing a microarray screening experiment.

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 42), 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 Web Portal. To download the ProtoArray® Prospector software and manual, go to www.invitrogen.com/protoarray, and click on Online Tools tab.

ProtoArray® Human Protein Microarray

Introduction

The ProtoArray® Human Protein Microarray nc 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 Complete Kits include 2 ProtoArray® Human Protein Microarrays.

Human Protein Microarray Specifications

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

Dimensions:	1 inch x 3 inch (25 mm x 75 mm)
Material:	Glass slide coated with nitrocellulose membrane
Membrane Size:	20 mm x 60 mm
Membrane Properties:	Thickness: 15-20 µm; Pore Size: 0.2 µm

Each microarray has a barcode for tracking samples. The barcode is also used to retrieve array specific information from the ProtoArray® Central Portal (see page 42).

Array Specifications

The array specifications for the ProtoArray® Human Protein Microarray nc 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 nc, 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 20 columns**
Median Spot Diameter:	~150 µm
Spot Center to Center Spacing:	220 µm
Distance Between Subarrays:	100 µm
Replicates per Sample:	2
Total Human Proteins on v4.0 array:	~8000*

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

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ProtoArray® Human Protein Microarray, Continued

Array Content

The majority of 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 250 proteins printed on the array are derived from the purified protein kinase collection 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 Information File from www.invitrogen.com/protoarray as described on page 42.

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 a 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.

After verifying that each clone expresses a protein of the expected molecular weight by western blotting, the proteins are expressed and purified using high-throughput procedures. The expressed proteins are purified by affinity chromatography under conditions optimized to obtain maximal protein integrity, function, and activity.

Note

Approximately 5000 human proteins printed on the ProtoArray® Human Protein Microarray nc v4.0 were also present on the previous version of the product, ProtoArray® Human Protein Microarray nc v3.0. However, not all proteins printed on ProtoArray® Human Protein Microarray nc v3.0 are also printed on ProtoArray® Human Protein Microarray nc v4.0. To obtain a list of ProtoArray® Microarray nc v3.0 human proteins that are not printed on ProtoArray® Microarray nc v4.0, contact Technical Support (page 54).

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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 12.

Printing the Human 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® Microarrays.

Maintaining Stringent Quality Control

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. 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 control proteins to detect the appropriate protein-protein interactions.

For detailed product qualification, see page 55.

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 nc to confirm the reciprocal interaction.

For example, perform an initial probing with calmodulin as a probe with a ProtoArray® Human Protein Microarray nc 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® Yeast Proteome Microarray

Introduction

The ProtoArray® Yeast Proteome Microarray nc v1.1 is a high-density protein microarray containing the majority of proteins from *S. cerevisiae*. Each *S. cerevisiae* open reading frame (ORF) is expressed as an N-terminal GST-6xHis fusion protein, purified, and printed in duplicate on a nitrocellulose-coated glass slide. This section provides details about the yeast proteome microarray including array specifications and preparation of proteins.

Note: The ProtoArray® Yeast Proteome Microarray PPI Complete Kit includes 2 ProtoArray® Yeast Proteome Microarrays.

Yeast Proteome Microarray Specifications

The specifications for the ProtoArray® Yeast Proteome Microarray nc v1.1 are listed below.

Dimensions:	1 inch x 3 inch (25 mm x 75 mm)
Material:	Glass slide coated with nitrocellulose membrane
Membrane Size:	20 mm x 60 mm
Membrane Properties:	Thickness: 15-20 µm; Pore Size: 0.2 µm

Each microarray has a barcode for tracking samples. The barcode is also used to retrieve array specific information from the ProtoArray® Central Portal (see page 42).

Array Specifications

The array specifications for the ProtoArray® Yeast Proteome 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 yeast protein and control spots on the ProtoArray® Yeast Proteome Microarray nc, go to the ProtoArray® Central Portal at www.invitrogen.com/protoarray.

Note: The subarray layout and controls are different in ProtoArray® Yeast Proteome Microarray nc v1.1 as compared to the previously available ProtoArray® Yeast Proteome Microarray nc v1.0.

Total Subarrays:	48 (4 columns x 12 rows)
Subarray Size:	4400 µm x 4400 µm
Subarray Dimensions:	**16 rows x 20 columns**
Median Spot Diameter:	~150 µm
Spot Center to Center Spacing:	220 µm
Distance Between Subarrays:	100 µm
Replicates per Sample:	2
Total Yeast Proteins on v1.1 array:	>4000*

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

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ProtoArray® Yeast Proteome Microarray, Continued

Preparing Yeast Proteins

The yeast proteome collection is derived from the *S. cerevisiae* clone collection of 5800 yeast ORFs (Zhu *et al.*, 2001). Each *S. cerevisiae* open reading frame (ORF) is expressed as an N-terminal GST-6xHis fusion protein in the yeast expression vector pEG-KG (Mitchell *et al.*, 1993). The identity of each clone is verified using 5′-end sequencing and the expression of GST-tagged fusion protein by each clone is confirmed with Western immunodetection using an anti-GST antibody. The proteins are then expressed and purified using high-throughput procedures.

Briefly yeast stocks are initiated in growth media, protein expression is induced with galactose, and cell lysates prepared. The proteins are purified using glutathione affinity chromatography, eluted, and purified proteins are used to spot the proteome microarray.

Printing the Yeast ProtoArray®

The purified yeast proteins are printed on nitrocellulose-coated slides in a dust-free, temperature, and humidity controlled environment to maintain consistent quality of microarrays. The arrays are printed using an automated process on an arrayer that is extensively calibrated and tested for printing ProtoArray® Microarrays.

Controls

Various proteins and controls are printed on each ProtoArray® Yeast Proteome Microarray to allow you to verify background and detection conditions during probing. For details, see page 12.

Detecting Reciprocal Interactions

ProtoArray® Yeast Proteome 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® Yeast Proteome Microarray nc, use the identified interacting protein from the array as a probe for to probe another ProtoArray® Yeast Proteome Microarray to confirm the reciprocal interaction. For an example of reciprocal interaction, see page 49.

Maintaining Stringent Quality Control

ProtoArray® Yeast Proteome Microarrays are produced using the same rigorous production and pre-printing and post-printing quality control procedures used to produce the human protein microarrays (see page 8). For detailed product qualification, see page 55.

Continued on next page

ProtoArray® Control Protein Microarray

Introduction

The ProtoArray® Control Protein Microarray nc 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 or Yeast Proteome Microarray.

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

Control Microarray Specifications

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

Dimensions:	1 inch x 3 inch (25 mm x 75 mm)
Material:	Glass slide coated with nitrocellulose membrane
Membrane Size:	20 mm x 60 mm
Membrane Properties:	Thickness: 15-20 µm; Pore Size: 0.2 µm

Each microarray has a barcode for tracking samples. The barcode is also used to retrieve array specific information from the ProtoArray® Central portal (see page 42).

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 yeast protein and control spots on the ProtoArray® Control Protein Microarray nc, 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:	**8 rows x 20 columns**
Median Spot Diameter:	~150 µm
Spot Center to Center Spacing:	220 µm
Distance Between Subarrays:	100 µm
Replicates per Sample:	2

Continued on next page

ProtoArray® Control Protein Microarray, Continued

Controls Printed on Each ProtoArray® Microarray

Various proteins and controls are printed on each ProtoArray® Human Protein, Yeast Proteome, and Control Protein Microarray nc 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.
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.
Control Spots NOT required for PPI Data Analysis	
Fiduciary Kinases	Kinases autophosphorylate and produce fiduciary (marker) signals which are used for orientation of the microarray image; also serves as a positive control for the radiolabel and assay conditions.
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.

ProtoArray® Control Protein Microarray, Continued

Note

The yeast calmodulin protein (Cmd1p; expressed as described on page 10) is printed on each microarray. When probing the ProtoArray® Control Protein Microarray nc 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 and yeast microarrays.

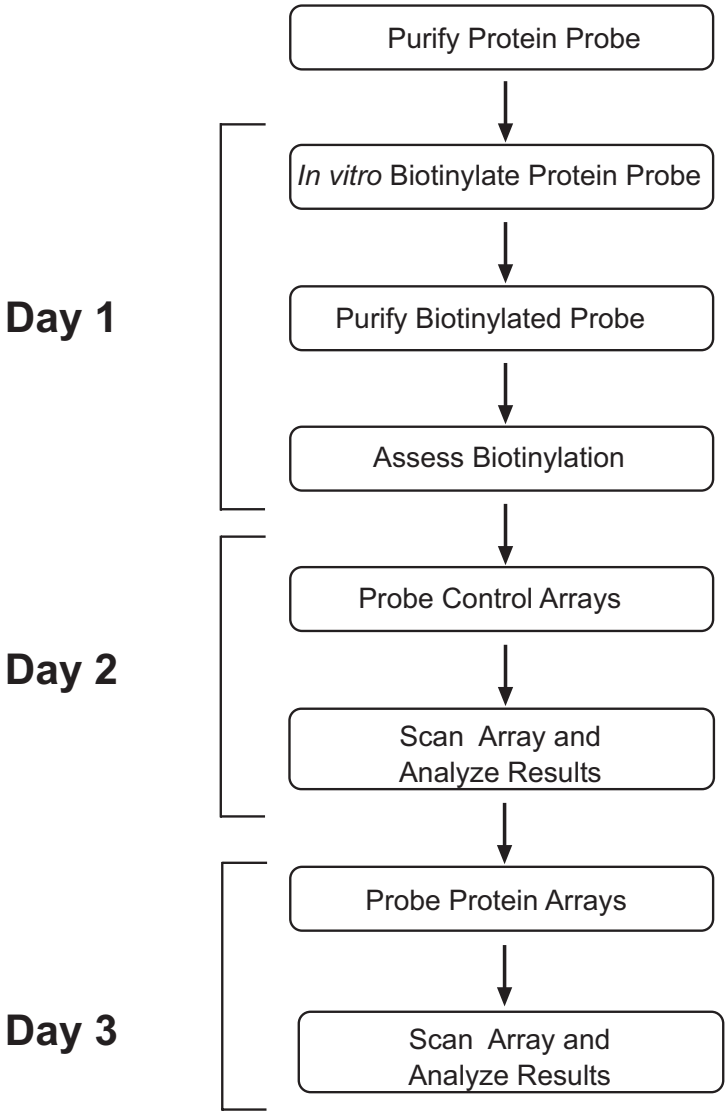
Maintaining Stringent Quality Control

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 and Yeast Proteome Microarrays (page 8). 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. For detailed product qualification, see page 55.

Experimental Overview

Experimental Timeline

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

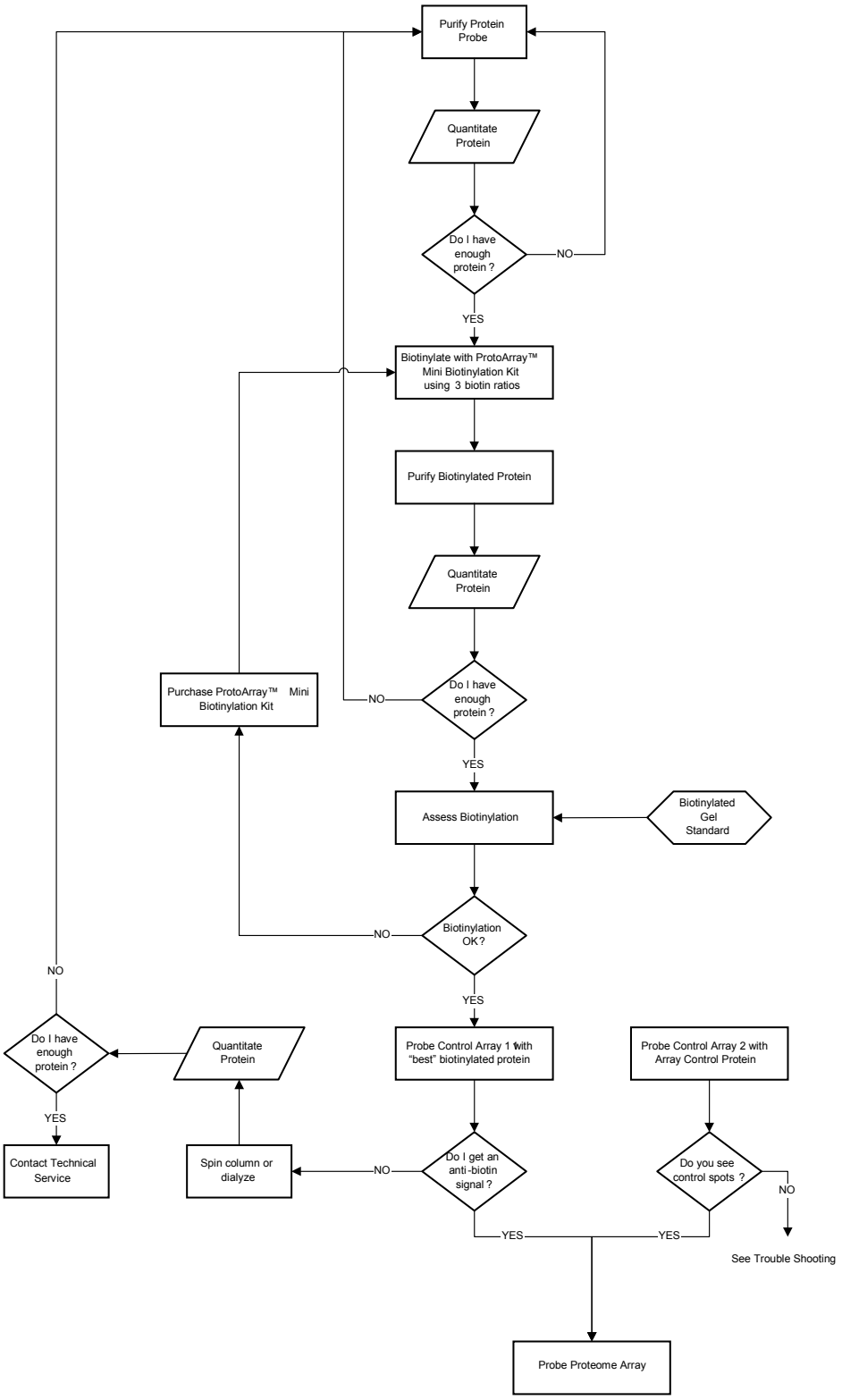


Continued on next page

Experimental Overview, Continued

Workflow

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



Methods

Preparing the Protein Probe

Introduction

Before using the ProtoArray® Human Protein or Yeast Proteome Microarray PPI Kit, you need your purified protein of interest to probe the microarray.

You may purify proteins using any method of choice. You can use proteins purified from *E. coli*, yeast cells, or higher eukaryotes to probe the ProtoArray® Microarray.

The amount of protein and quality of protein required for probing are described below.

Protein Quality

After you have expressed your protein of interest, follow the guidelines below to purify and prepare the protein probe.

- Purify the protein probe to > 90% purity as determined by Coomassie® staining.
- Resuspend the purified protein probe in a buffer ($\leq$ 50 mM) that does not contain any primary amines such as ammonium ions, Tris, glutathione, imidazole, or glycine. If the buffer contains primary amines, sufficiently dialyze proteins against 50 mM HEPES buffer, pH 7.4 containing 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.

Amount of Protein

You need at least 150 µg of purified protein at a concentration of 2.5 mg/ml.

In Vitro Biotinylation

Introduction

Instructions are provided in this section to biotinylate your protein probe with the ProtoArray® Mini-Biotinylation Module (supplied with the ProtoArray® complete kits). See the next page for an outline of experimental steps.

ProtoArray® Mini-Biotinylation Module

To obtain the best results with the ProtoArray® Human or Yeast PPI Kits, use the ProtoArray® Mini-Biotinylation Module supplied with the kit to biotinylate your protein probe. The module is specifically formulated to biotinylate small amounts of your protein probe at 3 different molar concentrations of biotin to protein and includes a control protein to verify biotinylation efficiency.

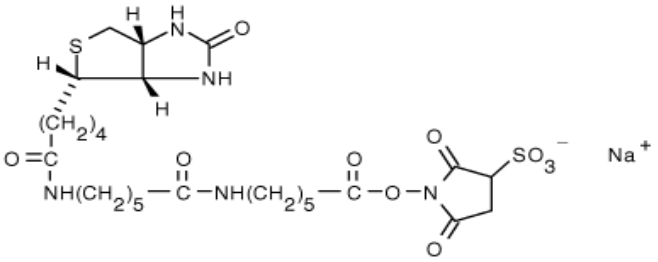
Biotin-XX Sulfosuccinimidyl ester, Sodium salt

The biotin-XX sulfosuccinimidyl ester, sodium salt included in the kit is water-soluble and readily reacts with the amine group of lysine residues to yield a biotin moiety covalently attached to the protein probe via two aminohexanoic chains ("XX"). This 14-atom spacer has been shown to enhance the ability of the biotin moiety to bind to avidin.

Molecular Formula: $C_{26}H_{40}N_5NaO_{10}S_2$

Molecular Weight: 669.74 Da

Structure of biotin-XX sulfosuccinimidyl ester, sodium salt



Biotin:Protein Ratios

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:

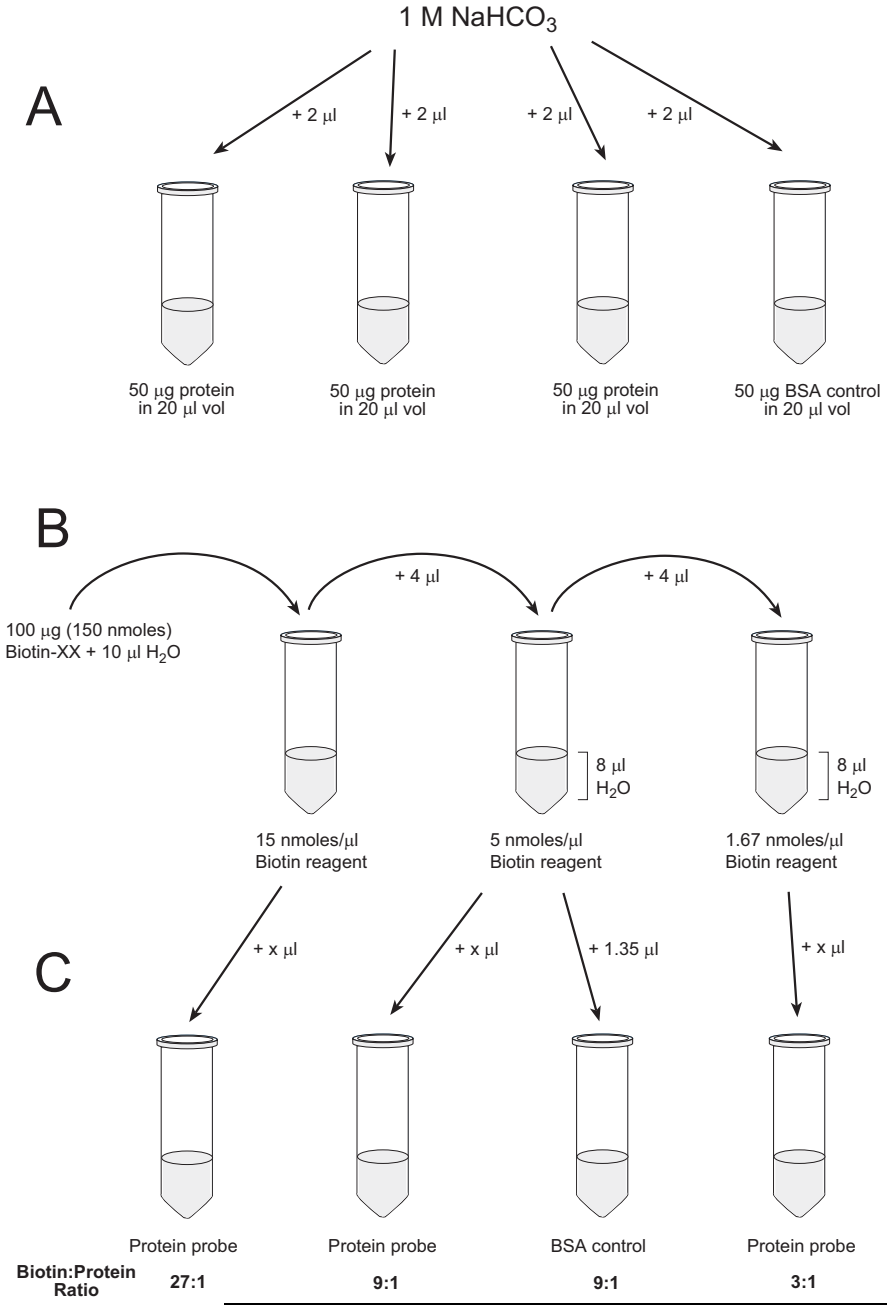
Molar Ratio	Biotin Molecules/Protein
3:1	1-2
9:1	3-5
27:1	10-15

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.

Continued on next page

Experimental Outline

The figure below outlines the steps required for *in vitro* biotinylation of your protein probe using the ProtoArray® Mini-Biotinylation Module.



1. Aliquot 20 µl of 2.5 µg/µl purified protein into 3 tubes and 20 µl of 2.5 µg/µl BSA control into 1 tube. Add 2 µl of 1 M $NaHCO_3$ into each tube.

2. Calculate the amount of biotin reagent (x µl) to add to your protein using the formula provided on the next page.

3. Prepare 3-fold serial dilutions of biotin reagent.

4. Add x µl of biotin reagent from each serial dilution to the appropriate protein sample to obtain the specified ratios of biotin:protein. Add 1.35 µl of 5 nmoles/µl biotin to the BSA control sample.

Continued on next page

In Vitro Biotinylation, Continued

Materials Needed

- Purified protein probe (page 16 for amount and quality of protein)
- Biotin-XX sulfosuccinimidyl ester, sodium salt (included in the ProtoArray® Mini-Biotinylation Module)
- Sterile water (included in the ProtoArray® Mini-Biotinylation Module), thaw and keep on ice until use
- BSA Control Protein (included in the ProtoArray® Mini-Biotinylation Module)
- Sterile microcentrifuge tubes
- **Freshly prepared** 1 M sodium bicarbonate buffer (see below for a recipe)

Preparing Buffer

Prepare 1 M sodium bicarbonate **fresh** prior to use. Dissolve 0.84 g of $NaHCO_3$ in 10 ml deionized water. The pH should be ~8.3–8.5.

Preparing Protein Samples

Prepare protein samples for biotinylating at 3 molar ratios (see Fig. A, previous page) and BSA Control Protein (included in the kit) as follows:

1. To 0.5 ml microcentrifuge tubes, add the following on ice:

Components	27:1	9:1	3:1	BSA
Protein Probe (2.5 mg/ml)	20 µl	20 µl	20 µl	--
BSA Control Protein (2.5 mg/ml)	--	--	--	20 µl
1 M Sodium Bicarbonate Buffer	2 µl	2 µl	2 µl	2 µl

2. Mix well and centrifuge briefly in a microcentrifuge at maximum speed.
3. Check the pH of the solution as incorrect pH may result in failed biotinylation. Spot a small amount of the mixture (1 µl) from Step 1 on a 7-10 pH paper and compare color with sodium bicarbonate buffer. The pH of the solution should be ~8.0.

   If the pH is not ~8.0 discard samples and dialyze purified protein against PBS. Prepare samples as described in Step 1 with dialyzed samples.

Calculating the Amount of Biotin Reagent

A formula is included below to calculate the amount of biotin reagent required for protein biotinylation in Step 6, next page. If you are an experienced user and are familiar with protein molar calculations, you may use your own calculation.

Use the formula below to calculate the amount of biotin reagent to use:

$$\frac{90{,}000}{\text{MW (Da)}} = \text{µl biotin reagent to be added to protein samples}$$

MW is the molecular weight of the protein probe in Daltons.

Example:
The molecular weight of your protein sample is 50,000 Da. Calculate the amount of biotin reagent required as follows:

$$\frac{90{,}000}{50{,}000} = 1.8 \text{ µl biotin reagent to be added to protein samples}$$

Continued on next page

Important

The reactive form of biotin-XX sulfosuccinimidyl ester rapidly hydrolyzes in water. Prior to dissolving biotin-XX sulfosuccinimidyl ester in water:

- Label and set up the dilution tubes as described below on ice
- Calculate the amount of biotin reagent required as described on the previous page
- Review the experimental outline on page 18

Dissolve biotin-XX sulfosuccinimidyl ester in cold water **immediately** before use and add the biotin reagent to the protein within **5 minutes** of initial resuspension of the reagent in water.

Biotinylation Reaction

The ProtoArray® Mini-Biotinylation Kit contains 100 µg (150 nmoles) of lyophilized biotin-XX sulfosuccinimidyl ester. Prepare and dilute the biotin reagent for biotinylating proteins at 3 molar ratios (see Fig. B, page 18) as follows:

1. To three 0.5 ml microcentrifuge tubes add the following on ice:

Tube	1	2	3
Chilled Sterile Water	--	8 µl	8 µl

2. To 100 µg (150 nmoles) of lyophilized biotin-XX sulfosuccinimidyl ester, add 10 µl cold sterile water.
3. Mix well by pipetting up and down. Be sure to completely dissolve the contents of the vial. Centrifuge briefly in a microcentrifuge. Transfer entire contents (10 µl) of the tube into Tube 1 from Step 1.
4. Transfer 4 µl from Tube 1 to Tube 2 containing 8 µl sterile water. Mix well and centrifuge briefly in a microcentrifuge.
5. Transfer 4 µl from Tube 2 to Tube 3 containing 8 µl sterile water. Mix well and centrifuge briefly in a microcentrifuge.

   The final concentration and volume in each tube is listed below:

Tube	1	2	3
Final Biotin Reagent Concentration	15 nmole/µl	5 nmole/µl	1.67 nmole/µl
Final Volumes	6 µl	8 µl	12 µl
Use for protein molar ratio	27:1	9:1	3:1

6. Add x µl (see formula on previous page to calculate the biotin reagent amount) from each tube from Step 5 to the 3 protein sample tubes prepared as described on previous page. See Fig. C, page 18.

   Add 1.35 µl Biotin Reagent (5 nmole/µl; from Step 5, tube 2) to the tube containing BSA Control Protein.

   The total volume in each tube = 22 µl protein + x µl biotin reagent
7. Mix well by pipetting and centrifuge briefly in a microcentrifuge. Avoid mixing the samples by vortexing.
8. Incubate at room temperature for 60 minutes. Proceed immediately to **Purifying Biotinylated Protein** (next page).

Purifying Biotinylated Protein

Introduction

This section provides instructions to purify the biotin-labeled protein from free biotin using the ProtoArray® Biotinylation Purification Module. The presence of free biotin in protein samples interferes with array probing and results in high background on the microarray.

Materials Needed

- Biotinylated samples (see previous page)
- ProtoArray® Biotinylation Purification Module (included in the ProtoArray® complete kit)

Experimental Outline

1. Purify biotinylated protein probe using gel filtration.
2. Estimate protein concentration of the purified biotinylated protein.

Purification Module

The ProtoArray® Biotinylation Purification Module includes a gel filtration resin (exclusion limit: 6,000 Da) and spin columns for rapid and efficient removal of free biotin from biotin labeled proteins. The expected yield of the biotinylated protein is 50-60%.

Preparing Spin Columns for Purification

While the biotinylation reaction is in progress, prepare the spin columns for purification as follows:

1. Remove 4 spin column/collection tube assemblies from the module.
2. Resuspend the Purification Resin by gently rocking the bottle until the resin is evenly suspended in the buffer. Avoid mixing by shaking or vortexing.
3. Transfer 800 µl of the Purification Resin into each of the 4 spin columns.
4. Centrifuge spin columns in a microcentrifuge at maximum speed (10,000-15,000 x g) for 15 seconds at room temperature.

   Note: The fixed angle rotor of a microcentrifuge causes the resin in the spin column to have a low side and high side.
5. Discard collection tubes and place spin columns in clean collection tubes (supplied in the module). Cap spin columns until sample application to prevent drying of resin, which affects recovery.
6. Proceed to the **Purification Procedure**, next page.

Continued on next page

Purifying Biotinylated Protein, Continued

Purification Procedure

At the end of the biotinylation incubation period (Step 8, page 20), purify the biotinylated samples using the spin columns prepared as described on the previous page.

1. Load each of the 4 biotinylation reactions (Step 8, page 20) onto 4 spin columns containing the purification resin prepared as described on previous page. Load samples onto the middle of the resin.
2. Place the spin column in the microcentrifuge with the high side of the resin pointing up.
3. Centrifuge the spin columns in a microcentrifuge at maximum speed (10,000-15,000 x g) for 1 minute at room temperature.
4. **The purified biotinylated protein is in the collection tube.** The eluate volume is ~50-100 µl and recovery is ~50–60%.
5. Save a small aliquot for protein estimation and assessing biotinylation (next page). Store remaining samples at 4°C, -20°C, or -80°C (depending on your protein). Use the samples to probe the ProtoArray® Human Protein or Yeast Proteome Microarray nc after assessing the level of biotinylation.
6. Determine the final protein concentration of the biotinylated protein samples with 5-10 µl of the column eluate using a method of choice.
7. Proceed to **Assessing Protein Biotinylation**, next page.

Assessing Protein Biotinylation

Introduction

Due to the differences in protein (*e.g.* lysine residues) the level of biotinylation on each protein varies. To obtain the best results with the biotinylated protein probe for use with the ProtoArray® Human Protein or Yeast Proteome Microarray nc, it is important to determine and assess the level of biotinylation for your protein sample. Instructions for assessing protein biotinylation with Western blotting and chemiluminescent detection using the ProtoArray® Biotinylation Assessment Module are provided in this section.

ProtoArray® Biotinylation Assessment Module

The ProtoArray® Biotinylation Assessment Module provides an efficient and sensitive method of assessing the level of biotinylation and includes a Biotinylation Gel Standard.

Assessment is performed by SDS-PAGE of biotinylated protein samples and the Biotinylated Gel Standard, Western transfer to nitrocellulose membranes (see **Note** below), detection with Streptavidin-AP conjugate, and visualization using a chemiluminescent substrate. The band intensities of the biotinylated protein samples are compared to the Biotinylation Gel Standard to assess the level of biotinylation.

Experimental Outline

1. Perform SDS-PAGE using biotinylated protein samples and the Biotinylation Gel Standard from the kit.
2. Transfer proteins to nitrocellulose membrane.
3. Perform Western chemiluminescent detection with Streptavidin-AP conjugate.
4. Verify and assess the level of biotinylation for your protein probe.

Note

We recommend using nitrocellulose membranes for Western detection to assess protein biotinylation. Our results with ProtoArray® Biotinylation Assessment Module have demonstrated lower sensitivity and higher background using PVDF membranes for Western detection.

Pre-Cast SDS-PAGE Gels

A large variety of pre-cast gels for SDS-PAGE are available from Invitrogen. For details, visit our web site at www.invitrogen.com or contact Technical Support (page 54).

We recommend using NuPAGE® Novex® Bis-Tris Gels and instructions are provided in this section to prepare samples for SDS-PAGE with NuPAGE® Gels.

If you are using Tris-Glycine gels, prepare samples as directed on pages 24-25. Run gels using the buffers and conditions recommended by the manufacturer.

Continued on next page

Assessing Protein Biotinylation, Continued

Materials Needed

- ProtoArray® Biotinylation Assessment Module (supplied with the complete kit)
- Biotinylation Gel Standard (supplied with the complete kit)
- Aliquot of purified biotinylated protein probe and BSA (step 6, page 22)
- 1X NuPAGE® LDS Sample Buffer (supplied with the complete kit)
- 10X NuPAGE® Reducing Agent (supplied with the complete kit)
- 2 NuPAGE® Novex® Bis-Tris Gels (page x)
- NuPAGE® MES or MOPS SDS Running Buffer (page x)
- NuPAGE® Antioxidant (page x)
- Nitrocellulose membranes (page x)
- Electrophoresis and blotting apparatus (page x)
- Deionized water
- Heating block set at 70°C
- Appropriate staining container for Western blotting
- Molecular weight markers (page x)

Preparing Biotinylation Gel Standard For SDS-PAGE

Prepare the following dilutions of the Biotinylation Gel Standard to generate a standard curve for SDS-PAGE. Each µl of the Biotinylation Gel Standard contains 20 fmoles of biotin conjugated to BSA and is used for assessing biotinylation.

1. Thaw the Biotinylation Gel Standard, 1X NuPAGE® LDS Sample Buffer, and 10X NuPAGE® Reducing Agent.
2. Transfer 900 µl 1X NuPAGE® LDS Sample Buffer to a microcentrifuge tube. Add 100 µl 10X NuPAGE® Reducing Agent to the tube to generate 1X Gel Loading Buffer.
3. Transfer 40 µl Biotinylation Gel Standard to a microcentrifuge tube. Add 4 µl 10X NuPAGE® Reducing Agent and 36 µl 1X Gel Loading Buffer (from Step 2) to obtain a 10 fmoles/µl stock (total volume = 80 µl).
4. Starting with the 10 fmoles/µl stock, prepare 2-fold serial dilutions of the Biotinylation Gel Standard to obtain 5 fmoles/µl, 2.5 fmoles/µl, 1.25 fmoles/µl, and 0.625 fmoles/µl stocks. For each dilution, dilute the standard with 1X Gel Loading Buffer to a final volume of 40 µl.
5. Heat the samples at 70°C for 10 minutes.
6. Load 20 µl sample on a NuPAGE® Novex® 4-12% Bis-Tris Gel as described on page 26.

Using these samples allows analysis of 200 fmoles, 100 fmoles, 50 fmoles, 25 fmoles, and 12.5 fmoles of the Biotinylated Gel Standard.

Continued on next page

Assessing Protein Biotinylation, Continued

Note

A formula is included below for your convenience to generate a stock solution for each of your protein samples after column purification. If you are an experienced user and are familiar with protein molar calculations, you may use your own method for calculation.

Formula for Generating Stock Solution

Use the formula below to calculate the final volume of the sample to generate a 200 fmoles/µl stock solution from 1 µl of column purified material for each of the 3 protein biotinylation reactions (treated at 27:1, 9:1, and 3:1 molar ratio) and control BSA biotinylation reaction (treated at 9:1 molar ratio).

You will need to know the protein concentration in mg/ml and the approximate molecular weight for each protein sample. The molecular weight of BSA (used for control biotinylation reaction) is 66,700 Da.

$$\frac{5 \times 10^6 \times \text{protein concentration (mg/ml)}}{\text{MW (Da)}} = \text{final volume in µl}$$

MW is the molecular weight of the protein in Daltons.

Example:
If the protein concentration of your sample after column purification (page 22) is 0.5 mg/ml and the MW of your protein is 50,000 Da, calculate the final volume as follows:

$$\frac{5 \times 10^6 \times 0.5}{50000} = 50 \text{ µl}$$

In this example, dilute 1 µl of each sample with 49 µl 1X Gel Loading Buffer to generate a **200 fmoles protein/µl** stock solution for each sample.

Preparing Biotinylated Protein Samples for SDS-PAGE

Prepare the following dilutions of the biotinylated protein sample and BSA Control Protein after column purification for assessing biotinylation.

1. Prepare a 200 fmoles/µl stock solution for each sample using the formula described above.
2. From the 200 fmoles/µl stock solution for each sample, prepare the following dilutions:
   - Dilute 1 µl of 200 fmoles/µl solution from each sample with 39 µl 1X Gel Loading Buffer to generate a **5 fmoles/µl** sample (total volume is 40 µl).
   - Dilute 10 µl of 5 fmoles/µl solution from each sample with 30 µl 1X Gel Loading Buffer to generate a **1.25 fmoles/µl** sample (total volume is 40 µl).
3. Heat the samples at 70°C for 10 minutes.
4. Load 20 µl sample on a NuPAGE® Novex® 4-12% Bis-Tris Gel as described on the next page. The final amount for each sample is listed on the next page.

Continued on next page

Gel Electrophoresis

After preparing samples, perform SDS-PAGE. You need 2 NuPAGE® Novex® Bis-Tris mini-gels for analysis.

Note: You may load samples on one gel as shown in the example on page 29 if desired.

The recommended loading pattern and final amount for each sample is listed below. Load 20 µl of each sample on the gel and 10 µl of a molecular weight protein standard. For samples containing 25 fmoles (Gel 1, Lanes 6-9) or 100 fmoles (Gel 2, Lanes 6-9) of biotinylated protein or BSA, use the 1.25 fmoles/µl or 5 fmoles/µl stock solutions (Step 2, page 25), respectively.

Gel 1

Lane	Sample	Final Amount in Gel
1	Biotinylated Standard	200 fmoles biotin
2	Biotinylated Standard	100 fmoles biotin
3	Biotinylated Standard	50 fmoles biotin
4	Biotinylated Standard	25 fmoles biotin
5	Biotinylated Standard	12.5 fmoles biotin
6	Biotinylated protein (3:1)	25 fmoles protein
7	Biotinylated protein (9:1)	25 fmoles protein
8	Biotinylated protein (27:1)	25 fmoles protein
9	Biotinylated BSA (9:1)	25 fmoles protein
10	Molecular Weight Standard	--

Gel 2

Lane	Sample	Final Amount in Gel
1	Biotinylated Standard	200 fmoles biotin
2	Biotinylated Standard	100 fmoles biotin
3	Biotinylated Standard	50 fmoles biotin
4	Biotinylated Standard	25 fmoles biotin
5	Biotinylated Standard	12.5 fmoles biotin
6	Biotinylated protein (3:1)	100 fmoles protein
7	Biotinylated protein (9:1)	100 fmoles protein
8	Biotinylated protein (27:1)	100 fmoles protein
9	Biotinylated BSA (9:1)	100 fmoles protein
10	Molecular Weight Standard	--

For NuPAGE® Novex® Bis-Tris Gels, perform SDS-PAGE at 200 V for 35-50 minutes using NuPAGE® MES or MOPS Running Buffer with an XCell *SureLock*™ Mini-Cell. Remember to include NuPAGE® Antioxidant in the running buffer (see instructions included with the NuPAGE® gels). After electrophoresis is complete, proceed to blotting, next page.

Continued on next page

Assessing Protein Biotinylation, Continued

Protein Transfer

Transfer proteins from the two gels to nitrocellulose membranes using a suitable transfer apparatus.

Note: PVDF membranes are not recommended (page 23) for use in Western transfer when using this protocol.

For NuPAGE® Novex® Bis-Tris Gels, perform transfer at 30 V for 1 hour using 1X NuPAGE® Transfer Buffer with 10% methanol.

After transfer, proceed to detection and visualization as described below.

General Guidelines

To obtain the best detection results with reagents included in the ProtoArray® Biotinylation Assessment Module, follow these guidelines:

- Use a single, clean container for each blot.
- Avoid touching the working surface of the membrane, even with gloves.
- Avoid cross-contamination of system solutions especially with the alkaline phosphatase substrate solution.
- Perform all washing, blocking, and incubation steps on a rotary shaker rotating at 1 revolution/second.
- Add solutions to the trays slowly, at the membrane edge, to avoid bubbles forming under the membrane. Decant from the same corner of the dish to ensure complete removal of previous solutions.

Preparing Solutions for Nitrocellulose Membranes

Prepare the solutions for analyzing 2 nitrocellulose membranes using the reagents included in the kit as described below.

Solution	For Nitrocellulose Membrane	
Blocking Solution	Ultra filtered Water	28 ml
	Western Blocking Solution A	8 ml
	Western Blocking Solution B	4 ml
	Total Volume	40 ml
Streptavidin-AP Solution (1:4000)	Streptavidin-AP Conjugate	5 µl
	Blocking Solution (above)	to 20 ml
Wash Solution	Ultra filtered Water	150 ml
	Western Washing Buffer (16X)	10 ml
	Total Volume	160 ml
Chemiluminescent Substrate	Chemiluminescent Substrate	4.75 ml
	Chemiluminescent Substrate Enhancer	0.25 ml
	Total Volume	5 ml

Continued on next page

Assessing Protein Biotinylation, Continued

Western Detection Protocol

1. Place each membrane in 10 ml of the Blocking Solution in a staining container. Incubate for 30 minutes on a rotary shaker set at 1 revolution/sec. Decant the Blocking Solution.
2. Rinse the membrane with 20 ml of water for 5 minutes, then decant. Repeat once.
3. Incubate the membrane in 10 ml of Streptavidin-AP Solution (1:4000) for 30 minutes, then decant.
4. Wash the membrane for 5 minutes with 20 ml of Wash Solution, then decant. Repeat 3 times.
5. Rinse the membrane with 20 ml of water for 2 minutes, then decant. Repeat twice.
6. Place the membrane on a sheet of transparency plastic. Do not allow the membrane to dry out.
7. With a clean pipette, evenly apply 2.5 ml of the Chemiluminescent Substrate to the membrane surface without touching the membrane surface. Let the reaction develop for 5 minutes.
8. Blot the excess Chemiluminescent Substrate solution from the membrane surface with the filter paper. **Do not allow the membrane to dry out.**
9. Cover the membrane with another clean piece of transparency plastic to prepare a membrane sandwich for luminography. Expose an X-ray film (we recommend Kodak X-OMAT AR films) to the membrane sandwich for 5-60 seconds (see next page for an example of the blot).

   Note: The alkaline phosphatase-activated CDP-Star® produces a maximum light emission wavelength at 466 nm to 461 nm, depending on the membrane environment of the reaction.
10. Proceed to assess the Western detection results, below.

Assessing Biotinylation

1. Verify that the protein sample and Control Protein (BSA) is biotinylated. You can also perform a densitometry scan. See next page for an example of a Western blot.
2. Compare the band intensities of 3 different molar ratios of biotinylated protein samples from Step 9, above to the BSA Control Protein and Biotinylation Gel Standard on the blot.
3. Use the biotinylated protein sample that gives the best signal at the lowest biotinylation molar ratio to probe the control array (page 30) and human or yeast array (page 36).

   The next page shows results of a biotinylation experiment and provides guidelines on interpreting your biotinylation results.

To troubleshoot biotinylation problems, see page 50.

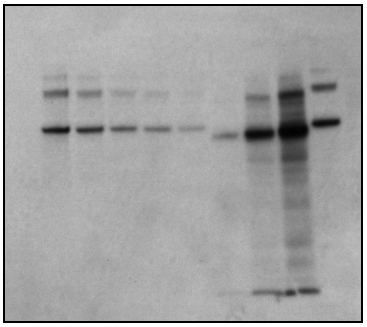
Continued on next page

Assessing Protein Biotinylation, Continued

Expected Results

An example of the Western blot of Biotinylation Gel Standard and a biotinylated protein probe (calmodulin kinase) is shown below.

Calmodulin kinase and Control Protein (BSA) were biotinylated as described in this manual. 20 µl of sample was electrophoresed on a NuPAGE® Novex® 4-12% Bis-Tris Gel using NuPAGE® MES SDS Running Buffer. The proteins were transferred to a 0.45 µm nitrocellulose membrane and proteins were detected using chemiluminescent detection as described on page 28 using a 5 second exposure.



Samples on the gel are:

Lane 1:	SeeBlue® Plus2 Pre-Stained Standard (10 µl)
Lanes 2-6:	Biotinylated Gel Standard 200, 100, 50, 25, and 12.5 fmoles, respectively.
Lane 7:	Calmodulin kinase (treated at 3:1 molar ratio), 25 fmoles
Lane 8:	Calmodulin kinase (treated at 9:1 molar ratio), 25 fmoles
Lane 9:	Calmodulin kinase (treated at 27:1 molar ratio), 25 fmoles
Lane 10:	BSA Control Protein (treated at 9:1 molar ratio), 25 fmoles

Interpreting Results

Compare the band intensities of your biotinylated protein to the BSA Control Protein and Biotinylation Gel Standard as described below to select a properly biotinylated protein probe (~3-5 biotin molecules/protein).

The BSA Control Protein (25 fmoles, lane 10, above) is modified with 3-5 biotin molecules per polypeptide. Loading 25 fmoles BSA Control Protein is equivalent to loading 75-125 fmoles biotin. The band intensity of 25 fmoles BSA Control Protein is approximately similar to the band intensity of 100 fmoles Biotinylation Gel Standard (lane 3, above).

For a protein with average lysine content (~8%), biotinylating at a molar ratio of 9:1 usually incorporates 3-5 biotin molecules/protein. The band intensity of 25 fmoles of protein probe biotinylated at 9:1 (lane 8, above) should be approximately similar to the band intensity of BSA Control Protein (lane 10, above) or 100 fmoles Biotinylation Gel Standard (lane 3, above). Based on the biotinylation results of the example shown in the gel, you can use calmodulin kinase biotinylated at 9:1 molar ratio for probing experiments.

Probing the ProtoArray® Control Protein Microarrays

Introduction

The ProtoArray® Control Protein Microarray nc allows you to verify *in vitro* biotinylation labeling and probing conditions. Probe the ProtoArray® Control Protein Microarrays **prior to** probing the ProtoArray® Human Protein or Yeast Proteome 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 complete kits 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 HybriSlip™ cover slips that hold a small reagent volume to minimize the amount of valuable probe used and prevent evaporation of reagents. Array Chambers are also included in the module for washing the microarrays.

ProtoArray® Application Kit

The ProtoArray® Protein-Protein Interaction Application Kit includes ProtoArray® PPI Buffer Modules A and B and the Alexa Fluor® detection reagent. The use of application kit provides consistent results and eliminates the time required to prepare reagents.

The ProtoArray® PPI Buffer Modules A and B include qualified reagents for blocking, washing, and probing during the microarray probing procedure (see above).

Before using the ProtoArray® Application Kit, you also need to purchase a ProtoArray® Human, Yeast, or Control Protein Microarray nc.

Materials Needed

- 2 ProtoArray® Control Protein Microarrays nc v4.0 (included in the complete kits only or 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 Probing Buffer (see next page)
- Array Control Protein in Probing Buffer (included in the complete kits only; see next page)
- 2 sterile 50 ml conical tubes
- 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.

Continued on next page

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 to avoid any damage to the array surface resulting 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 and immersing the array immediately in blocking solution equilibrated at 4°C
- Perform array experiments at a clean location to avoid dust or contamination and filter solutions if needed (particles invisible to eyes can produce high background signals and cause irregular spot morphology)
- Avoid drying the array during the experiment and ensure the array is completely covered with the appropriate reagent during all steps of the protocol
- Always dry the array prior to scanning and 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** (from Step 3, page 28): 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

Array Control Protein

The Array Control Protein (included in the complete kits) 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 nc, 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

Prepare the following buffers **fresh** prior to use. The recipe below provides sufficient buffer to probe 1 microarray. To probe 2 microarrays, scale up the amount of reagents used accordingly.

PBST Blocking Buffer

1X PBS
1% BSA
0.1% Tween 20

1. Use reagents provided in the kit to prepare 30 ml PBST Blocking Buffer as follows:

ProtoArray® Blocking Buffer (10X)	3 ml
30% BSA	1 ml
Deionized water	to 30 ml

2. Mix well (do not vortex) and store on ice until use.

Probing Buffer

1X PBS
5 mM $MgCl_2$
0.5 mM DTT
0.05% Triton X-100
5% Glycerol
1% BSA

1. Use reagents provided in the kit to prepare 180 ml Probing Buffer as follows:

ProtoArray® Probe Buffer (5X)	36 ml
1 M DTT	90 µl
1 M $MgCl_2$	0.9 ml
30% BSA	6 ml
Deionized water	to 180 ml

2. Mix well (do not vortex) and store on ice until use.

After preparing PBST Blocking Buffer and Probing Buffer, immediately return the remaining 5X ProtoArray® Probe Buffer, 10X ProtoArray® Blocking Buffer, and 1 M $MgCl_2$ to 4°C, and 30% BSA and 1 M DTT to -20°C.

Continued on next page

Probing the ProtoArray® Control Protein Microarrays, Continued

Preparing the Probes

Array Control Protein (biotinylated calmodulin kinase)
Mix 12 µl of the Array Control Protein (included in the complete kits) with 120 µl of Probing Buffer. 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 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 (previous page), biotinylated probes in Probing Buffer (previous page), Array Chambers (included in the kit), and HybriSlips™ (included in the kit).
- Make sure the buffers are cold. Store buffers on ice until use. Place the Array Chambers on ice to chill the chamber until use.
- Review **Important Guidelines** on page 31 prior to starting the probing procedure.

Blocking Step

Instructions for blocking the ProtoArray® Control Protein Microarray nc are described below:

1. Remove the mailer containing the ProtoArray® Control Protein Microarray nc from storage at -20°C and immediately place the mailers at 4°C.
2. Allow the mailer containing the array to equilibrate at 4°C for at least 15 minutes prior to performing the blocking step.
3. Perform blocking in the mailer. Ensure that the microarray is placed properly in the chamber with the printed (white) side facing up. Add 30 ml PBST Blocking Buffer (page 32) to the chamber containing the array.

   Note: You can block 2 arrays simultaneously in the mailer using 30 ml PBST Blocking Buffer.
4. Incubate for 1 hour in the cold room with gentle shaking (~50 rpm).
5. Decant the PBST Blocking Buffer. Drain excess buffer by inverting the mailer on paper towels for a few seconds. Remove the array from the mailer. Tap one edge of the array gently on a laboratory wipe for a few seconds to drain any buffer **without allowing the array to dry**. Place the array on a clean, flat surface with the printed side of the array facing up.
6. Proceed immediately to **Probing the Array**, next page.

Continued on next page

Probing Control Array

1. Pipet 120 µl of the biotinylated protein probe (50 µg/ml) in Probing Buffer (page 32) on top of one control array without touching the array surface. Add 120 µl Array Control Protein (50 µg/ml) in Probing Buffer on top of the second ProtoArray® Control Protein Microarray nc. The liquid quickly spreads over the nitrocellulose membrane.
2. Carefully lift the HybriSlip™ cover slip from the support liner with forceps and lay the clear side of HybriSlip™ on 1 array to cover the membrane area without trapping any air-bubbles. The HybriSlip™ is designed to exactly cover the membrane area. Gently adjust the HybriSlip™ to remove any air bubbles. Repeat for the second array. Insert each assembly (array with HybriSlip™) into a separate 50 ml conical tube with the printed side of the array facing up. Cap the conical tube.
3. Place the conical tubes on a flat surface such that the printed side of the array is facing up and the tubes are as level as possible. If needed, you can tape the conical tubes on a flat surface to avoid any accidental disturbances. Incubate the arrays in the tube for 1.5 h at 2-8°C **without shaking**.
4. Remove each array from the conical tube and insert diagonally (see **Note** below) into an Array Chamber kept on ice.

   Note: The microarray with HybriSlip™ does not fit on the rails of the chamber. You must insert the microarray diagonally into the chamber.
5. Using a sterile pipette, add 25 ml Probing Buffer (page 32) to the chamber wall while keeping the chamber on ice. **Avoid pipetting buffer directly onto the array surface.** Gently move the array in the chamber to dislodge the HybriSlip™.
6. Using forceps, carefully remove each HybriSlip™ without touching the array surface. Do not remove the HybriSlip™ with the forceps if the HybriSlip™ is not dislodged from the array. Discard the HybriSlip™. Reposition the array on the chamber rails, if desired.
7. Incubate the array in Probing Buffer for ~1 minute on ice. Decant the Probing Buffer. Invert chamber on paper towels for a few seconds to drain excess buffer.
8. Repeat Step 7 two more times, using 25 ml Probing Buffer each time.
9. While the array is incubating, prepare Streptavidin-Alexa Fluor® 647 Conjugate solution by mixing 6 µl of the Streptavidin-Alexa Fluor® 647 Conjugate (included with the kit) with 25 ml Probing Buffer.
10. After the third wash with Probing Buffer (Step 8), decant the buffer. Invert chamber on paper towels for a few seconds to drain excess buffer. Add 25 ml of the Streptavidin-Alexa Fluor® 647 Conjugate from Step 9 to the chamber.
11. Incubate the chamber for 30 minutes on ice in the dark (cover the ice bucket). Decant the buffer. Invert the chamber on paper towels for a few seconds to drain excess buffer.
12. Slowly add 25 ml Probing Buffer onto the chamber wall while keeping the chamber on ice. Avoid pipetting buffer directly onto the array surfaces.
13. Incubate the array in Probing Buffer for ~1 minute on ice. Decant the buffer. Drain excess buffer by inverting chamber on paper towels for a few seconds.
14. Repeat Steps 12-13 two more times, using 25 ml Probing Buffer each time.
15. Proceed to **Drying the Arrays**, next page.

Continued on next page

Probing the ProtoArray® Control Protein Microarrays, Continued

Drying the Arrays

1. Remove the arrays from the chamber at the end of the probing procedure. Tap one edge of each array gently on a laboratory wipe for a few seconds to drain any buffer.
2. Place the arrays in a slide holder (or a sterile 50 ml conical tube, if you do not have a slide holder) in a vertical orientation. Ensure that the arrays are properly placed and secure in the holder to prevent any damage to the arrays during centrifugation.
3. Centrifuge the arrays in the slide holder or 50 ml conical tube at 800 x g for 3-5 minutes in a centrifuge (equipped with a plate rotor, if you are using the slide holder) at room temperature.
4. Place the arrays in a slide box and keep the box with the lid open in the **dark** for 30-60 minutes at room temperature to dry the arrays. Make sure the array is completely dry; there should be no translucent areas.
5. Scan the array using a fluorescence microarray scanner (see page 41 for details).

Data Analysis

After scanning and saving an image of each array, analyze results to identify positive interactors. For more details, see page 42.

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 42.
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 44 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 46. For troubleshooting, see page 50.
4. After confirming the appropriate interactions on the Control Arrays, proceed to **Probing the ProtoArray® Human or Yeast Microarrays**, next page.

Cleaning the Chamber

At the end of probing experiments, clean the Array Chambers properly and rinse with sterile water before re-using the chambers.

Probing the ProtoArray® Human or Yeast Microarrays

Introduction

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

Materials Needed

- ProtoArray® Human Protein Microarray nc **or** ProtoArray® Yeast Proteome Microarray nc (included in the complete kit)

  Note: If you have purchased the ProtoArray® Protein-Protein Interaction Application Kit, you also need to purchase the ProtoArray® Human Protein or Yeast Proteome Microarray nc separately.
- ProtoArray® PPI Buffers Module A and B (included in the kit)
- Your biotinylated protein probe in Probing Buffer (see next page)
- Streptavidin-Alexa Fluor® 647 conjugate (keep on ice in the dark until immediately before use)
- Sterile 50 ml conical tube
- Ice bucket
- Deionized water
- *Optional*: Microarray slide holder and centrifuge equipped with a plate holder

Important

Each ProtoArray® Human Protein or Yeast Proteome Microarray nc 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 or Yeast Proteome Microarray nc.
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 31 to obtain the best results with the arrays.

Continued on next page

Probing the ProtoArray® Human or Yeast Microarrays,
Continued

Probes for Proteome Arrays

The ProtoArray® Human Protein or Yeast Proteome Microarray PPI Complete Kit contains 2 human or yeast arrays, respectively, and can be probed using the different probing options as 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 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

Preparing Buffers

Prepare PBST Blocking Buffer and Probing Buffer as described on page 32.

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 (see previous page), probes in Probing Buffer (see previous page), Array Chambers (included in the kit), and HybriSlips™ (included in the kit).
- Make sure the buffers are cold. Store buffers on ice until use. Place the Array Chamber on ice to chill the chamber until use.
- Review **Important Guidelines** on page 31 prior to starting the probing procedure.

Continued on next page

Probing the ProtoArray® Human or Yeast Microarrays,
Continued

Probing Arrays

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

1. Probe the ProtoArray® Human Protein or Yeast Proteome Microarray nc using the procedure described on page 33.
2. Dry the array as described on page 35.
3. Scan the arrays as described on the next page and analyze results (page 42).

Examples of expected results obtained after probing the ProtoArray® Human Protein or Yeast Proteome Microarrays are shown on pages 47 and 48, respectively.

If you obtain weak signals or high background, see **Troubleshooting**, page 50.

Scanning Arrays

Introduction

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

Materials Needed

You need a fluorescence microarray scanner to scan the ProtoArray® Human Protein or Yeast Proteome Microarray nc. To acquire ProtoArray® data from the image, you will need appropriate microarray data acquisition software.

The recommended microarray data acquisition software for analysis is GenePix® Pro (Molecular Devices Corporation) or ScanArray® Software (PerkinElmer, Inc.).

The scanner specifications are listed below and recommended scanners are listed on the next page.

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 or Yeast Proteome Microarray nc 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 ($\pm$ 200 µm)
	Excitation	635 nm or equivalent
	Detection limit	0.1 fluor/µm^2
	Resolution	$\leq$10 µm
	Dynamic Range	>3 orders of magnitude
	Output	16-bit TIFF

Recommended Scanners

The following scanners are **compatible** for scanning ProtoArray® Human Protein or Yeast Proteome Microarrays:

- 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 or Yeast Proteome Microarrays:

- 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 or Yeast Microarrays:

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

Note

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

Continued on next page

Scanning Procedure

A brief procedure for scanning the ProtoArray® Human Protein or Yeast Proteome Microarrays 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 or Yeast Proteome Microarray nc 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 area for record) 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 is included in the name of the image.

   Note: Examples of expected image scans of control, human, and yeast arrays are shown on pages 46-49.
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 Online Tools.

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, Yeast, 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 slide.

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

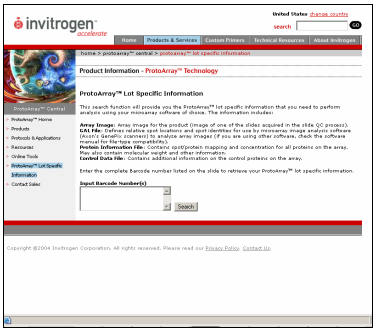
Note: The .GAL files are text files that contain the data in a format specified by GenePix® Pro Microarray data acquisition software. If you are using any other microarray data acquisition software, you can use data from the .GAL files to generate files that are compatible with your microarray data acquisition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

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

1. Go to www.invitrogen.com/protoarray and then click on the Online Tools tab.
2. Click on the link to ProtoArray® Lot Specific Information.
3. Enter the array barcode in the Input Barcode Number(s) box. Click on the Search button.



Continued on next page

ProtoArray® Central, continued

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.

   Protein Application File (LotNumber_application.PAI):

   ProtoArray® Prospector uses the Protein Application Files for data analysis. Different PAI files are designed for different applications. For example, ProtoArray® Prospector uses the file HA10756 PPI.PAI to analyze data from PPI experiments performed on array from lot HA10756.

   Slide Information File (LotNumber_slide.txt):

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

5. Download the files listed above for human or yeast array-specific information from a specific lot. Use these files to interpret your results with the ProtoArray® Human Protein or Yeast Proteome Microarray nc as described below.

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

6. Start the microarray data acquisition software on the computer and open the saved image (.tiff) from Step 8, page 41.

7. 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.

Continued on next page

ProtoArray® Central, continued

8. 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 (next page). 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.

Alternatively, save/export the results with an .xls extension or rename the .tab or .gpr file using the .xls extension for data analysis using Microsoft® Excel.

Analyzing Data

After data acquisition, analyze the data to identify protein interactions.

Visual identification of interactions can be performed after initial identification of significant interactions is done using the data analysis guidelines listed below.

We recommend using the ProtoArray® Prospector software available from Invitrogen for data analysis. This software allows rapid data analysis without the need to perform any manual calculations. For more information, see below.

Performing the data analysis by importing the data file into Microsoft® Excel or an equivalent spreadsheet program to identify potential substrates is not recommended. This approach requires a certain degree of expertise with statistics and Excel or another spreadsheet program.

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 slides.

The ProtoArray® Prospector software and manual are available free-of-charge to ProtoArray® users. To download the ProtoArray® Prospector software or manual, go to www.invitrogen.com/protoarray, and click on the Online Tools tab. Install the **Basic** version of ProtoArray® Prospector for data analysis.

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 protein probe. If .GPR files are not available, consult the ProtoArray® Prospector 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.

Based on the Z-score and available protein sequence information, 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 or Yeast Proteome Microarray nc, 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 or Yeast Proteome Microarray nc using a similar or a different probe concentration to observe similar interactions.
- **Specificity:** Probe a ProtoArray® Human Protein or Yeast Proteome Microarray nc 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 49 for an example).

Other methods for validating protein-protein interactions include:

- Yeast Two-Hybrid Systems (page x)
- 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 54) to order the purified protein kinases printed on the array.

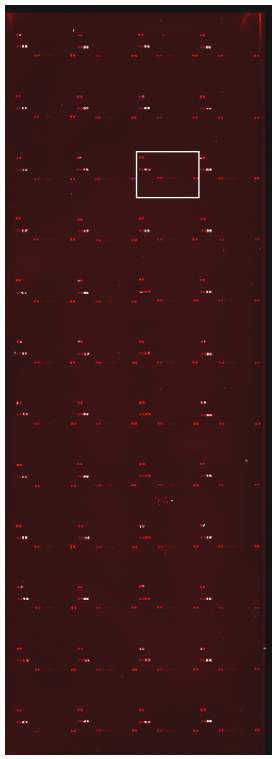
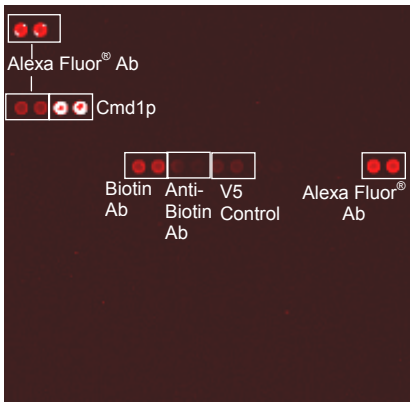
Note: The yeast proteins printed on the array are derived from the Snyder collection (Zhu *et al.*, 2001). For information on obtaining the yeast clone corresponding to the potential protein identified on the array, contact Technical Support (page 54).

Expected Results

Control Array Probing Results

Results obtained after probing the ProtoArray® Control Protein Microarray nc v4.0 with the Array Control Protein (*i.e.* BioEase™-V5-tagged biotinylated calmodulin kinase) are shown below.

Image showing calmodulin (Cmd1) signal when probed with 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.
- 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.
- Cmd1p signal
  The Array Control Protein (BioEase™-V5-calmodulin kinase) binds to the calmodulin printed on the array. The signal is used to verify the probing procedure.
- V5 Control signal
  The V5 control protein contains an N-terminal BioEase™ and V5 epitope tag, and binds to the Streptavidin-Alexa Fluor® 647 conjugate.

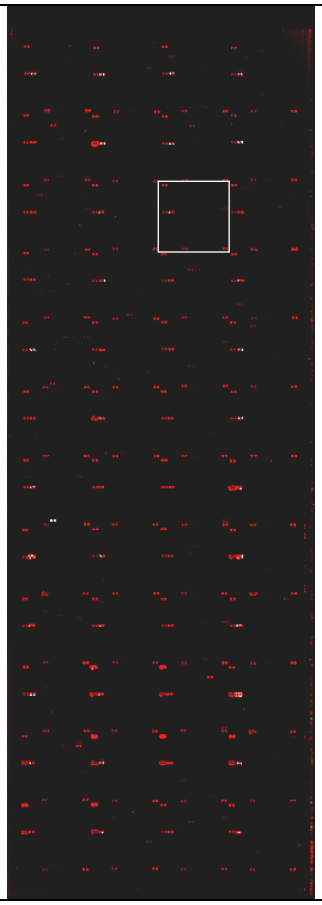
Continued on next page

Expected Results, Continued

Human ProtoArray® Probing Results

The results obtained after probing the ProtoArray® Human Protein Microarray nc v4.0 with 50 µg/ml of the Array Control Protein (*i.e.* BioEase™-V5-tagged biotinylated calmodulin kinase) probe is shown below.

Image showing Array Control Protein (probe) interacting with Cmd1p on the Human Microarray nc v4.0

Array Image	Boxed Area shown in detail
	

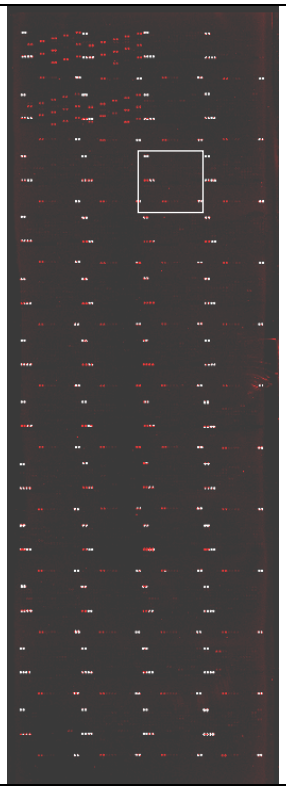
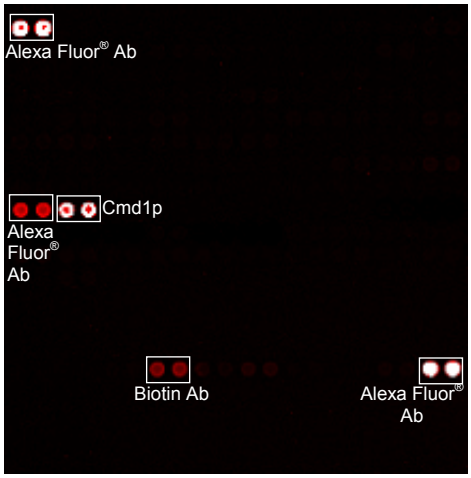
Continued on next page

Expected Results, Continued

Yeast ProtoArray® Probing Results

The results obtained after probing the ProtoArray® Yeast Proteome Microarray nc v1.1 with 50 µg/ml of the Array Control Protein (*i.e.* BioEase™-V5-tagged biotinylated calmodulin kinase) probe are shown below.

Image showing the Array Control Protein probe interacting with calmodulin (Cmd1p) on the array

Array Image	Boxed Area shown in detail
	

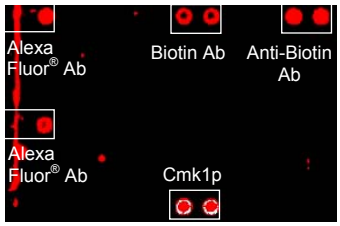
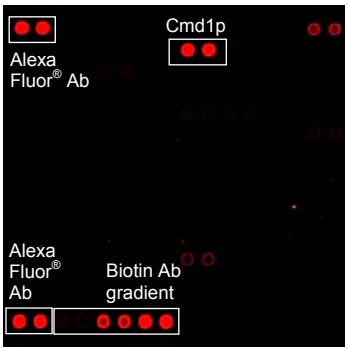
Note: The column of interactions observed on the top left corner for the array is due to calmodulin printed on the microarrays as control spots. The interaction of the yeast proteome calmodulin with the calmodulin kinase probe is shown in detail.

Continued on next page

Expected Results, Continued

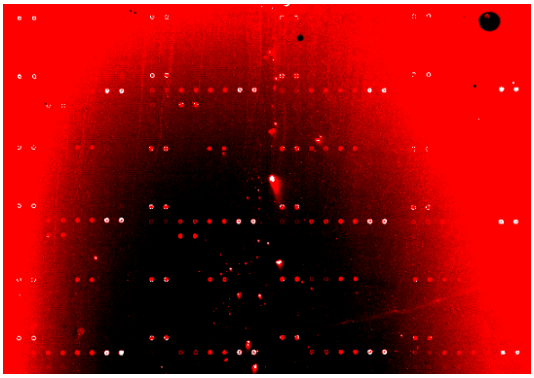
Example of Reciprocal Interaction

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 nc 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 or Yeast Proteome Microarray PPI Complete 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.
Only Biotinylated Gel Standard bands visualized	Poor or no biotinylation of BSA Control protein and protein probe	Make sure that the biotinylation reaction was performed as described on page 20 using the specified molar ratios and at pH ~8.0. Check that the calculations and serial dilutions were performed correctly.
		Check the pH after addition of sodium bicarbonate buffer to ensure the pH of the sample is ~pH 8.0.
		Add the Biotin-XX reagent to the protein probe within **5 minutes** as the reagent degrades quickly in an aqueous solution.
Only Biotinylated Gel Standard and Biotinylation Control Protein bands visualized	Poor or no biotinylation of your protein probe	Make sure the protein is in a buffer that does not contain any primary amines such as ammonium ions, Tris, glutathione, imidazole, or glycine.
		The Biotin-XX reagent degrades quickly in an aqueous solution and must be added to the protein probe within **5 minutes**.
		Make sure that the biotinylation reaction was performed as described on page 20 using the specified molar ratios and at pH ~8.0. Check that the calculations and serial dilutions were performed correctly.
	Protein has low lysine content or lysine residues are not readily available for biotinylation	Perform the biotinylation reaction at a 27:1 molar ratio or higher. You may express your protein as a fusion to a tag that contains lysine.
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.

Continued on next page

Problem	Cause	Solution
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 41) 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 PBST Blocking Buffer and Probing Buffer **fresh** as described on page 32.
		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.
High background	Improper blocking	Prepare the PBST Blocking Buffer **fresh** as described on page 32. Do not use the ProtoArray® Blocking Buffer included in the kit without the addition of BSA.
	Improper washing	For the best results, perform the recommended washing steps. Prepare the Probing Buffer **fresh** as described on page 32.
	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 35 before scanning.

Continued on next page

Problem	Cause	Solution
Control Array Results, continued		
Uneven background	Uneven blocking or washing	During the blocking or washing steps, ensure the array is completely immersed in PBST Blocking solution or Probing Buffer and use at least 30 ml buffer in the Array Chamber to cover the array completely with buffer.
	Improper washing	To obtain the best results, perform the recommended washing steps. Prepare the Probing Buffer **fresh** as described on page 32.
	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 Array Chamber to avoid scratching the array surface.
	Biotinylated protein probe not applied properly	Apply the probe solution and HybriSlip™ to the array as described in the manual. To avoid drying of the membrane, make sure the HybriSlip™ covers the membrane area of the array and adjust the HybriSlip™, if needed.
	Probe or detection reagents contain precipitates	Centrifuge the probe or detection reagents to remove precipitates prior to probing the array.
Human Protein or Yeast Proteome Array Results		
Weak or no signal with biotinylated protein probe	Poor biotinylation of protein probe	See page 37 for details.
	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.
		Use the recommended settings (page 41) to obtain a good image.
	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.

Continued on next page

Problem	Cause	Solution
Human Protein or Yeast Proteome Array Results, continued		
High background	Improper blocking	Prepare the PBST Blocking Buffer **fresh** as described on page 32. Do not use the ProtoArray® Blocking Buffer included in the kit without the addition of BSA.
	Improper washing	To obtain the best results, perform the recommended washing steps. Prepare the Probing Buffer **fresh** as described on page 32.
	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 35 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 or yeast 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.

Signal Due to Interaction with Detection Reagent

The following yeast protein produces a significant signal with the Streptavidin-Alexa Fluor® 647 conjugate. For a list of proteins that may interact with Alexa Fluor® 647 conjugated-streptavidin, see the ProtoArray® Central Portal.

ORF Name

YGL062W

Note that the signal does not indicate a positive interaction. The array yeast protein is biotinylated based on the E-motif database; therefore, it produces signal with Streptavidin-Alexa Fluor® 647 conjugate.

For more information on the E-motif database, visit:
http://db.yeastgenome.org/cgi-bin/SGD/protein/getDomain?sgdid=S0003030

Appendix

Technical Support

Web Resources

Visit the Invitrogen Web site 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

Contact Us

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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MSDS

MSDSs (Material Safety Data Sheets) are available on our website at www.invitrogen.com/msds.

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Product Qualification

Introduction

The components supplied in the ProtoArray® Human Protein and Yeast Proteome Microarray PPI Complete Kits are qualified as described below.

ProtoArray® Human, Yeast, and Control Microarrays

The ProtoArray® Human Protein, Yeast Proteome, and Control Microarrays are visually examined for obvious defects. The quality of the printing process is verified by probing several arrays from each lot with an anti-GST antibody. The scanned image of the array must show a uniform spotting pattern. The arrays are also functionally qualified by probing with the Array Control Protein (biotinylated calmodulin kinase) probe to ensure that appropriate interactions and controls are detected.

Array Control Protein

The Array Control Protein (biotinylated calmodulin kinase) is qualified by performing a Western detection with streptavidin-AP, and must show that the protein is biotinylated. The protein concentration of the Array Control Protein must be within the specified range.

ProtoArray® PPI Buffers Module

The 10X Blocking Buffer and 5X Probe Buffer are diluted to 1X with deionized water and subjected to pH and conductivity measurements. The pH and conductivity for each buffer must be within the specified range.

ProtoArray® Mini Biotinylation Module

Biotin-XX Sulfosuccinimidyl Ester

The Biotin-XX sulfosuccinimidyl ester must be >90% pure as analyzed by TLC and NMR analysis must indicate the correct structure.

Biotinylated Gel Standard (BSA)

MALDI-TOF (Matrix Assisted Laser Desorption/Ionization-Time Of Flight) analysis must indicate the specified moles of biotin per mole of BSA.

ProtoArray® Biotinylation Purification Module

The Purification Resin is qualified by loading 50 µg BSA solution and measuring the recovery. The BSA recovery must be >35 µg. The resin must also meet specifications for flow rate, fractionation range and particle size.

Continued on next page

ProtoArray® Biotinylation Assessment Module

The ProtoArray® Biotinylation Assessment module is qualified as follows:

- The Western chemiluminescent detection reagents in the module are qualified by performing electrophoresis of human IgG on a NuPAGE® Novex® 4-12% Bis-Tris Gel using NuPAGE® MES SDS Running Buffer. After electrophoresis, protein is transferred onto a nitrocellulose membrane using NuPAGE® Transfer Buffer. Immunodetection is performed as described in this manual using anti-human IgG primary antibody. 10-100 pg antigen must be detected within 30 minutes of exposure.
- The Streptavidin AP conjugate is tested in a Western blot with 12.5 fmoles of biotin conjugated to BSA. A signal must be obtained using a 1:4000 dilution of Streptavidin-AP and detection with a chemiluminescent substrate after a 30 second exposure to film.

Streptavidin-Alexa Fluor® 647 Conjugate

The spectra for the Streptavidin-Alexa Fluor® 647 conjugate must indicate an absorption maxima 654 $\pm$ 5 nm and emission maxima of 669 $\pm$ 5 nm. The degree of labeling is verified and must be 2.5-4 moles of Alexa Fluor® 647 dye per mole of protein. The conjugate is functionally qualified by immunocytochemistry and must show good nuclear staining with negligible background.

Purchaser Notification

Introduction

Use of the ProtoArray® Human Protein or Yeast Proteome Microarray PPI Complete Kit for Epitope-Tagged Proteins is covered under the licenses described below.

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Continued on next page

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