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ProtoArray® Control Protein Microarray v4.1 for Kinase Substrate Identification (KSI)

Catalog No. PA10012 **Quantity: 1 array** **Store at –20ºC**

Introduction

The ProtoArray® Control Protein Microarray v4.1 for KSI is used to verify probing and detection conditions using radiolabeled ATP prior to actual experiments with the ProtoArray® Human Protein Microarray v4.1 for KSI. Each array contains kinase substrates and various controls printed on a nitrocellulose-coated glass slide. Instructions are included in this section for probing the ProtoArray® Control Protein Microarray v4.1 using a control kinase (*e.g.* MAPK14).

For detailed instructions, microarray specifications, ProtoArray® technology overview, troubleshooting, and license information, download the **ProtoArray® Applications Guide** from www.invitrogen.com.

Contents and Storage

Each ProtoArray® Control Protein Microarray v4.1 box contains a mailer with one control protein microarray. Upon receipt, **store the microarray at –20°C.** Use the array before the expiration date printed on the packaging for best results.

Experimental Overview

1. Block the ProtoArray® Control Protein Microarray v4.1 with Blocking Buffer.
2. Probe array with kinase and [γ^{33}P]ATP, then wash to remove free [γ^{33}P]ATP.
3. Dry the array for imaging.
4. Expose the array to a phosphorimager screen and scan the screen to obtain an array image.
5. Download lot specific protein array information from the ProtoArray® Central portal and acquire the image data using microarray data acquisition software.
6. Analyze results using ProtoArray® Prospector data analysis software available from www.invitrogen.com/protoarray.

Important Guidelines

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

- **Do not** use the ProtoArray® Control Protein Microarray for detecting kinase-substrate interaction with your specific kinase of interest. The Control Protein Microarray **does not** contain the entire set of proteins printed on the ProtoArray® Human Protein Microarray for KSI.
- The ProtoArray® Control Protein Microarray can only be used once. **Do not re-use or re-probe** the array.
- Always wear clean gloves while handling microarrays.
- **Do not** use [γ^{32}P]ATP in place of [γ^{33}P]ATP, as data quantitation with [γ^{32}P]ATP is not supported.
- **Do not** touch the surface of the array. Damage to the array surface can result in uneven or high background.
- Maintain the array and reagents at 2–8°C during the experiment unless otherwise specified.
- Avoid drying of the array during the experiment. Ensure the array is completely covered with the appropriate reagent during all steps of the protocol.
- Perform array experiments at a clean location to avoid dust or contamination. Filter solutions as needed (particles invisible to the eye can produce high background signals and cause irregular spot morphology).
- Dry the array by centrifugation prior to exposing. **Do not** dry the array using compressed air or commercial aerosol sprays. Expose the array immediately upon completion of the experiment.

Working with Radioactive Materials

Follow these general guidelines when working with radioactive material. Refer to the ProtoArray® Applications Guide for additional details.

- Do not work with radioactive materials until you have been properly trained.
- Follow all the radiation safety rules and guidelines mandated by your institution.
- Wear protective clothing (laboratory coat, disposable gloves, and eyewear), and use a radiation monitor.
- Work in areas designated for radiation use, and monitor continuously for radioactive contamination.
- Dispose of radioactive waste properly. This includes reagents discarded during the probing procedure (*e.g.* washes).

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Materials Needed

- ProtoArray® Control Protein Microarray v.4.1 for KSI and buffers (see recipes page 2)
- Control kinase (MAPK14 (p38 alpha), active (Invitrogen, Cat. no. PV3304), recommended)
- [γ^{33}P]ATP (3,000 Ci/mmol, 10 µCi/µl)
- 0.45 µm filters (Millipore, Cat. no. SLHVR25LS)
- Clean, covered 4-chamber incubation tray (Greiner, Cat. no. 96077307 or ISC Bioexpress, Cat. no. T-2896-1) , chilled on ice
- Sterile 50 ml conical tubes
- Forceps and deionized water
- Shaker (capable of circular shaking at 50 rpm; place the shaker at 4°C)
- Incubator set to 30°C
- 60 × 24 mm glass coverslips (VWR, Cat. no. 48404-454)
- Microarray slide holder and centrifuge equipped with a plate holder (*Optional*)
- X-ray film cassette and clear plastic wrap
- Packard Cyclone Phosphorimager (Perkin-Elmer, Cat. no. B431200); Multisensitive Phosphor Screen (Perkin-Elmer, Cat. no. 7001723)
- Microarray data acquisition software (*e.g.* GenePix® Pro from Molecular Devices; refer to the ProtoArray® Applications Guide for details and optional software packages)
- Data analysis software (ProtoArray® Prospector available at www.invitrogen.com/protoarray, recommended)

Preparing Buffers

Prior to use, prepare the following buffers **fresh**. Mix all buffers well, sterile filter, and store on ice until ready for use.

Buffer	Composition	Preparation
Blocking Buffer 5 ml of buffer is needed for each microarray.	1X PBS, pH 7.4 1% BSA	1. Prepare 100 ml Blocking Buffer **fresh** as follows: 10X PBS, pH 7.4 — 10 ml 30% ***protease free*** BSA — 3.3 ml Deionized water — to 100 ml 2. Mix well and store on ice until use.
Kinase Buffer 120 µl of buffer is needed for each microarray.	1% NP-40 100 mM MOPS, pH 7.2 100 mM NaCl 1% BSA 5 mM $MgCl_2$ 5 mM $MnCl_2$ 1 mM DTT	1. Prepare 1 ml Kinase Buffer **fresh** as follows: 10% NP-40 — 100 µl 1 M MOPS, pH 7.2 — 100 µl 5 M NaCl — 20 µl 30% ***protease free*** BSA — 33 µl 1 M $MgCl_2$ — 5 µl 1 M $MnCl_2$ — 5 µl 1 M DTT — 1 µl Deionized water — to 1 ml 2. Filter buffer with 0.45 µm filter. Store at -20°C until use.
0.5% SDS 80 ml of buffer is needed for each microarray.	0.5% SDS	1. Prepare 200 ml 0.5% SDS **fresh** as follows: 10% SDS — 10 ml Deionized water — to 200 ml 2. Mix well and store at room temperature until use.

Control Kinase

A control kinase is needed to probe the ProtoArray® Control Protein Microarray v4.1 for KSI. A variety of purified kinases are available from Invitrogen for use with the ProtoArray® Control Protein Microarray. We recommend using MAPK14 (p38 alpha), active (see **Materials Needed**, above). It is also recommended to probe the array with your kinase of interest as a control to assess the compatibility of the kinase with the array surface and kinase performance under the specified assay conditions.

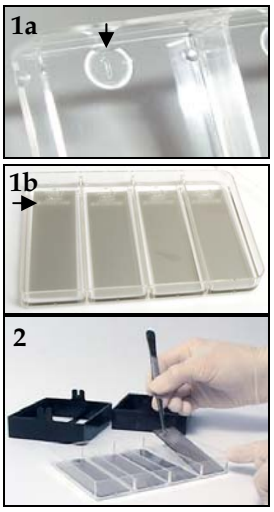
Preparing the Kinase

You need 120 µl Kinase Buffer containing a control kinase to probe **one** ProtoArray® Control Protein Microarray. If using your own kinase of interest, kinase activity and level of autophosphorylation will influence concentration. Too much kinase may result in a high background or a dark array, and too little kinase will result in no additional spots relative to a kinase-free control.

At Step 2 of the **Probing Procedure** 33 nM [γ^{33}P]ATP will be added to the diluted kinase. Once the ATP is added to the kinase, use the kinase-ATP mixture immediately for probing the array. Do not store the prepared kinase-ATP mixture on ice for more than 2 minutes prior to use on the array.

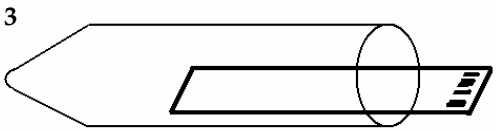
1. Prepare a 120 µl dilution of the control kinase, or your kinase of interest at a final concentration of 50 nM in Kinase Buffer.
2. Mix well (**do not** vortex) and store on ice until use. Immediately return the remaining kinase to –80°C.

Blocking Step



1. Remove the mailer containing the ProtoArray® Control Protein Microarray v4.1 from storage and place immediately at 4°C. Allow the array to equilibrate in the mailer at 4°C for at least 15 minutes before blocking. Not doing so may result in condensation on the array which can reduce protein activity or alter spot morphology.
2. Place one microarray with the barcode facing up into each well of a chilled 4-chamber incubation tray such that the barcoded end of the microarray is near the end of the tray marked with an indented numeral (see figures 1a and 1b).
3. Using a sterile pipette, add 5 ml Blocking Buffer into chamber containing the array. **Avoid pipetting buffer directly onto the array surface**.
4. Incubate the tray for 1 hour at 4°C on a shaker set at 50 rpm (circular shaking).
5. After incubation, remove array from 4-chamber incubation tray using forceps. Insert the tip of the forceps into the indentation at the numbered end of the tray and gently pry the array upward (see figure 2). Using a gloved hand, pick up the microarray by holding the array by its **edges** only. Tap to remove excess liquid from array surface.
6. Proceed immediately to the **Probing Procedure**, below.

Probing Procedure



1. Place the ProtoArray® Control Protein Microarray in a 50 ml conical tube with one-third of the slide extended outside of the tube (see figure 3). The barcode should be outside the tube, face up.
2. For each ProtoArray® Control Protein Microarray, add 1 µl of [γ^{33}P]ATP (3000 Ci/mmol, 10 µCi/µl) to 120 µl of Kinase Buffer containing diluted kinase (see **Preparing the Kinase**).
3. Pipet mixture gently onto the surface of the array in the conical tube.
4. Using forceps, carefully lay a glass coverslip on the surface of the microarray without trapping any air bubbles. Align the coverslip flush with the top edge of the array to ensure the printed area of the array is completely covered.
5. Position the coverslipped array so that it is inside the conical tube with the printed side (barcode) facing up, and cap the tube.
6. Place the conical tube horizontally on a flat surface in an incubator set to 30°C with the printed side of the array facing up and the tube as level as possible. If needed, tape the tube to the flat surface to avoid any accidental disturbances.
7. Incubate the conical tube containing the control array for 1 hour at 30°C **without shaking**.
8. Remove the conical tube containing the array from incubator and add 40 ml of 0.5% SDS to the tube by dispensing the SDS down the sides of the tube. **Avoid pipetting SDS directly onto the array surface**. The glass coverslip will float off. Remove glass coverslip from tube with forceps and discard as radioactive waste.
9. Cap tube and incubate at room temperature for 15 minutes **without shaking**. Discard the wash as radioactive waste.
10. Add 40 ml 0.5% SDS to the tube (dispense SDS as described in Step 8), cap tube, and incubate for 15 minutes **without shaking**. Discard the wash as radioactive waste.
11. Add 40 ml of water to the tube (dispense water as described in Step 8), and incubate for 15 minutes at room temperature **without shaking**. Discard the water wash as radioactive waste, and repeat the wash a second time.
12. Remove the array from the tube using forceps and place in a slide holder.
13. Proceed immediately to **Drying and Scanning the Microarray**, below.

Drying and Imaging the Microarray

1. Dry the control array using a table top centrifuge. Spin the array at 200 × g for 1–2 minutes at room temperature in the slide holder (if using a centrifuge equipped with a plate rotor) or 50 ml conical tube (if using a swinging bucket rotor). Verify that the array is completely dry.
2. Place control array in X-ray film cassette, cover with plastic wrap and overlay with phosphorscreen or X-ray film.
3. Expose control array to phosphorscreen or X-ray film for 6–36 hours.
4. Remove phosphorscreen from cassette and scan with phosphorimager or develop film using film developer.
5. Obtain 16-bit TIFF image file by scanning X-ray film with scanner or retrieving file from phosphorimaging of phosphor screen.

Protocol continued on next page.

Drying and Imaging the Microarray, continued

6. Process image using imaging software (*i.e.* Prospector Imager or Adobe® Photoshop®). For Prospector Imager, refer to the ProtoArray® Prospector User Guide. For Adobe® Photoshop® process the image as follows:
   a) Crop 1″ × 3″ fixed rectangular areas from each TIFF file that correspond to each slide.
   b) Invert data.
   c) Change image file to 2550 × 7650 pixels (constrained proportions).
   d) Save cropped TIFF image with new name.

   Note: Do not adjust pixel levels of file in Adobe® Photoshop® as this will affect the dynamic range of the spots.
7. Proceed to **Data Acquisition and Analysis**, below.

Data Acquisition and Analysis

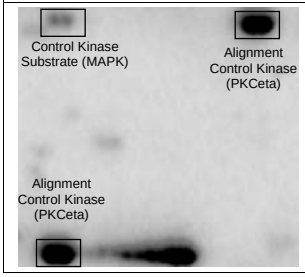
For data acquisition, download lot specific protein array information including the .GAL file from the ProtoArray® Central Portal as described in this section. The .GAL (GenePix Array List) files describe the location and identity of all spots on the microarray and are used with the microarray data acquisition software to generate files containing pixel intensity information for all features on the array.

1. Connect to the portal at www.invitrogen.com/protoarray and then click on the **ProtoArray® Lot Specific Information** link that can be found under **BioMarker Discovery Resources**.
2. Enter the array barcode in the **Input Barcode Number** box and click on the **Search** button.
3. For each input barcode, various lot specific files are displayed.
4. Start the GenePix® Pro microarray data acquisition software on the computer. Open the saved image (.tiff) from Step 6, above and open the .GAL files downloaded from ProtoArray® Central for protein arrays. The .GAL file defines the array grid required by the microarray data acquisition software.

   Important: Make sure you 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, download the latest version of lot specific information files.
5. Adjust the subarray grid to ensure the grid is in proper location for each subarray. After the grid is properly adjusted and all features are aligned, acquire the pixel intensity data for each feature by clicking the **Analyze** button in GenePix® Pro, and save/export the results as a .GPR (GenePix® Results) file.
6. Use the files from Step 5, above, for data analysis using ProtoArray® Prospector (available through the **Online Tools** link that can be found under **BioMarker Discovery Resources** at www.invitrogen.com/protoarray).
7. Install **complete** version of ProtoArray® Prospector.
8. Start ProtoArray® Prospector from the desktop icon. Set the **Application** to Kinase Substrate Identification.
9. Select the **Analyze** button from the Tool Bar.
10. Select the .GPR files from the "Files of type" pull-down list and navigate to your data file(s). Select the file(s) for analysis and click the **Open** button.
11. After analysis, ProtoArray® Prospector generates a list of human proteins showing significant interactions with the control kinase.

Expected Results

Results obtained after probing the ProtoArray® Control Protein Microarray with 50 nM of a MAPK14 (p38 alpha) control kinase and radiolabeled ATP are shown below. Refer to the ProtoArray® Applications Guide for additional details on control features.

Image	Control	Description/Function
	Alignment Control Kinases	Autophosphorylating kinase used for orientation of the microarray image, and serving as control for proper radiolabel and assay conditions.
	Control Kinase Substrate	Substrate for control kinase serves as control for proper probing and scanning procedures.

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This product is covered by Limited Use Label License. See the ProtoArray® Applications Guide or www.invitrogen.com for detailed information.