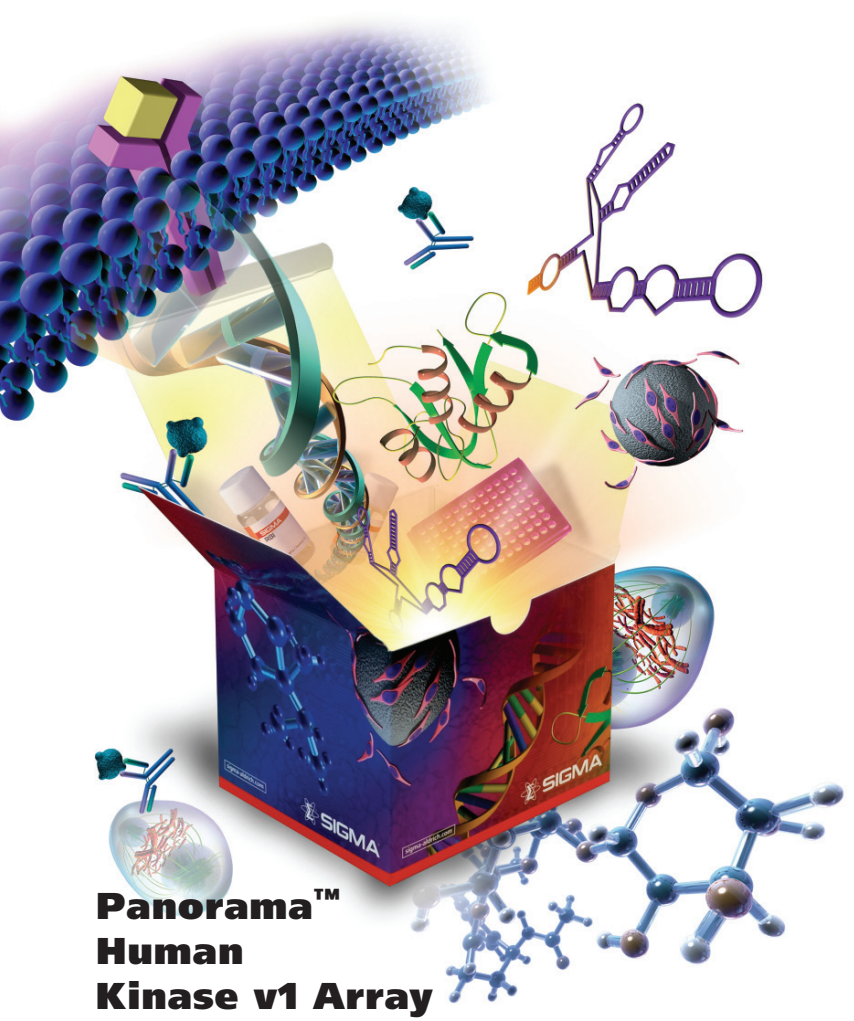


Panorama™ Human Kinase v1 Array

Technical Bulletin

Catalog Number
HPFM3

SIGMA-ALDRICH

Panorama™ Human Kinase v1 Array

Table of Contents

Introduction

The Panorama™ Human Kinase v1 Array contains 152 kinase proteins and specific controls. Kinases are categorized into famiies based on their structure and activity and are known to regulate the majority of cellular pathways, especially those involved in signal transduction and transmission of signals within the cell. Loss of kinase function has been linked to many human diseases, such as cancer[1], and is known to affect cascades of reversible phosphorylation. Kinases are attractive targets for therapeutic drugs because they have similar structures and highly conserved ATP binding sites. Drugs which inhibit specific kinase activity such as Gleevec® and Iressa® are currently in clinical use for the treatment of numerous malignancies.

Panorama Array Technology

The kinases on the Panorama Human Kinase v1 Array were expressed in Sf9 insect cells and affinity purified directly on the array via their biotin tag. As a result of the proprietary BCCP (biotin-carboxyl carrier protein) tagging technology, all kinases are presented in a similar orientation while providing a 50 Å spacer arm to maximize the opportunity for sites to interact with binding partners[2,3,4]. Open reading frames (ORFs) are cloned in frame with two tag sequences at the C terminus encoding the BCCP tag and the c-Myc epitope (EQKLISEEDL), which can be used to visualize and quantitate the proteins on the array. To ensure fidelity, clones are sequence-verified immediately prior to expression in Sf9 insect cells.

During expression, the BCCP tag is biotinylated only when it is correctly folded[5]. All expressed proteins are assayed for incorporation of biotin, and Western blot analysis is used to determine molecular weight, confirm biotinylation, and establish full-length protein has been expressed.

Biotinylation of BCCP occurs at a single surface-exposed lysine residue approximately 50 Å from the attachment to the fusion protein. The BCCP-biotin-fusion proteins are captured on the array surface via a streptavidin-biotin interaction with BCCP acting as a spacer between the array substrate and the fusion protein. BCCP-biotin provides a single-point high-affinity anchor so that all proteins on the array are in the same orientation. As a result, the arrayed proteins are not sterically or functionally hindered by multiple non-specific interactions with the surface and are freely available to interact with biochemical probes presented in solution, thereby minimizing non-specific interactions[6].

Panorama functional protein arrays are fabricated on borosilicate glass slides that display high chemical resistance, low auto-fluorescence, and excellent surface uniformity. The slides are cut by a laser to minimize particle contamination. The slides are then coated with streptavidin that is covalently attached to a permeable three-dimensional coating comprised of a cross-linked matrix with low non-specific protein-binding. The format is compatible with conventional microarray scanners and instrumentation.

Kit Contents

Product	Cat. No.	Size
Panorama Human Kinase v1 Microarrays	**P2374**	2 each
Anti-phosphotyrosine-Cy5	**T3576**	10 µL
Anti-c-Myc-Cy3	**C6594**	10 µL
Functional Assay Buffer	**C0492**	150 mL
Assay Buffer	**A1105**	150 mL
Bovine Serum Albumin (BSA)	**A3059**	2 × 200 mg
1M Dithiothreitol (DTT)	**646563**	1 ampule

Product	Cat. No.	Size
100 mM ATP	**A6559**	100 µL
quadriPERM® culture vessel	**Z376760**	2 each
HybriSlip™	**H0784**	10 each
Pap Jar	**P8123**	4 each
50 mL Conical Centrifuge Tubes	**C8296**	2 each
Panorama Kinase Array v1 Analysis Workbook and GAL file (CD)	**K4139**	1 each

Materials Required But Not Provided

- High-purity water
- Powder-free gloves
- Microarray scanner or fluorescence imager
- Microarray analysis software
- Forceps (fine and blunt-ended)
- Centrifuge
- Shaking incubator
- Orbital shaker
- Lint free tissue paper (e.g., Kimwipes)

Storage Conditions

Proteins on the array are sensitive to heat and oxidation. To preserve protein activity, the arrays are shipped on dry ice in screw-capped Pap jars and filled with 30 mL of storage buffer containing dithiothreitol and glycerol.

Upon receipt, store the kit at –20 °C until use. The storage buffer for the protein arrays may be frozen upon arrival due to the dry ice used for shipping, but will thaw gently when placed at –20 °C. Once the storage buffer has thawed, open Pap jars only prior to use.

General Recommendations

a. The array area covers most of the slide surface; therefore, extreme care is needed in handling the arrays. Remove the arrays from their storage buffer by the labeled end using blunt-ended forceps. Do not touch the unprotected portion of the slide surface.

b. Keep arrays in ice-cold buffer unless higher temperatures are required for assays.

c. Always keep the array label-side upwards when lying flat. To remove components of the storage buffer, wash arrays as indicated in the protocols.

d. Cover arrays completely in assay buffer/reagents to prevent them from drying out during the assay.

e. If using critical volume sample, use the HybriSlip provided with the kit. **Do not** use glass cover slips as they may sequester the sample. Pipette 50 µL of sample carefully onto the middle of the slide and lower the cover slip gently onto the surface using fine forceps.

   If sample is not limited and larger volume incubations are possible, perform incubations in plastic quadriPERM containers provided with the kit. Use sufficient sample solution to immerse the arrays. Assays in 2–5 mL of probing solutions yield the best results.

f. Protect fluorescent probes such as Cy-dye-labeled ligands from light during the assay. Cover the array with aluminum foil.

g. When compatible with assay conditions and detection methods, include 20% glycerol and 0.1% Triton X-100 in buffers.

h. The kinase arrays have been pre-blocked with 2% BSA during production. Additional blocking with non-fat milk powder or other commonly used blocking agents may be necessary.

i. If a high degree of background speckling is observed after processing, use despeckling algorithms that are present in most commercially available microarray analysis software. Please note that speckling affects the appearance of the slide image only and does not materially affect the quantitative data generated.

Kit Components

Protein Arrays

Each Kinase v1 array contains 152 protein kinases, numerous control features, and 48 markers in 48 sub-grids per slide. The protein kinases within the arrays have been printed in quadruplicate in 5 × 4 subarrays. Each feature on the array has a spot diameter of approximately 500 µm spaced at intervals of 300 µm. The arrayed kinase proteins are listed in Appendix B. Every fusion protein is expressed from a full-length ORF and each clone is fully sequence verified prior to protein expression. The sequence of the cloned ORFs and links to protein databases can be downloaded from the Sigma-Aldrich Web site (sigma-aldrich.com). A schematic and key to the Kinase v1 array are shown in Appendices A and B.

Each Kinase v1 array contains:

- 152 kinase proteins
- 48 Cy3/Cy5-labeled BSA marker spots for array alignment
- 4 negative control spots consisting of the immobilization tag, BCCP
- 4 negative control spots consisting of β-galactosidase-BCCP
- 4 anti-c-Myc positive control spots consisting of β-galactosidase-c-Myc
- 4 anti-c-Myc positive control spots consisting of β-galactosidase-BCCP-Myc
- Dilution series of 12 anti-c-Myc positive control spots consisting of β-galactosidase-BCCP-Myc
- Dilution series of 12 phosphotyrosine peptide control spots consisting of oriented peptides joined to the surface via a linker and consisting of the sequence, biotin-Ahx-KVEKIGEGT[pY]GVVKK-$CONH_2$, in which the tyrosine residue is phosphorylated.
- Dilution series of 12 kinase substrate control spots consisting of oriented peptides joined to the surface via a linker and consisting of the sequence biotin-Ahx-KVEKIGEGTYGVVKK-$CONH_2$, which acts as a substrate for certain tyrosine kinases.

- Dilution series of negative control spots consisting of biotin-BSA
- 36 sets of negative control spots printed with lysis buffer

The proteins are arrayed onto a streptavidin-coated, low fluorescence glass slide (25 × 75.6 × 1 mm) that is compatible with conventional microarray instrumentation. The upper side is indicated by the label (Appendix A). The arrays are pre-washed and blocked with 2% BSA to reduce non-specific binding. If additional blocking is desired, see Assay Guidelines for recommendations.

Anti-phosphotyrosine-Cy5

To validate the functionality of proteins on the Kinase v1 array, an autophosphorylation assay using Cy5-labeled anti-phosphotyrosine is supplied as a control. In the control assay, ATP is applied to the slide and phosphorylation of tyrosine is detected by the labeled monoclonal antibody. Although some proteins become phosphorylated when they are expressed in Sf9 cells, incubation of the array in buffer containing ATP leads to higher levels of phosphorylation of particular proteins (Figure 1). Autophosphorylation and detection should be carried out using the buffers provided with this kit.

Anti-c-Myc-Cy3

A Cy3-labeled anti-c-Myc monoclonal antibody is provided and serves as a positive control because the c-Myc tag is incorporated into all protein kinases on the array. It is recommended to use the anti-c-Myc to probe each control or experimental array immediately after performing primary assays for protein function. This assay demonstrates that proteins are immobilized at each spot and can be used to quantify the amount of protein for data normalization (Figure 2). Perform the binding assay with the Assay Buffer provided with the kit.

Control Assays

Protocol for Autophosphorylation Assay

a. Allow the Functional Assay Buffer to equilibrate to room temperature. In order to perform the assay, it is necessary to make 2 buffers from the Functional Assay Buffer.

b. Prior to performing the Autophosphorylation Assay, add 150 µL of 1 M DTT to the bottle containing 150 mL of Functional Assay Buffer and place at room temperature.

c. Prepare the Anti-phosphotyrosine Probing Buffer: add 200 mg of BSA to 10 mL of Functional Assay Buffer+DTT from step b. Mix gently to dissolve.

d. Prepare the **Phosphorylation Assay Buffer+ATP**: add 10 µL of 100 mM ATP solution to 10 mL of Functional Assay Buffer+DTT from step b. Mix gently by inversion.

e. Remove array from storage buffer and place in a clean Pap jar containing 25 mL of Functional Assay Buffer+DTT. Place the Pap jar on ice and shake gently (40–50 rpm) for 5 min.

f. Remove the array from the Functional Assay Buffer+DTT and drain the slide by blotting the long edge carefully onto lint-free tissue paper for 10 seconds.

g. Dry the back of the slide with lint-free tissue paper.

h. Place the slide into a chamber of a quadriPERM culture dish so that it is horizontal with array side up. Ensure the slide does not rest on the plastic lugs at the numbered end of the chamber as this may lead to incomplete coverage of the slide. Immediately pipette 5 mL of the Phosphorylation Assay Buffer+ATP carefully onto the slide, while minimizing introduction of bubbles. Add any additional slides to the chambers in the same manner, then replace the lid.

i. Place the chamber in a shaking incubator at 30 °C. Ensure the assay solution covers the entire array. Shake the dish gently for 30 min at 30 °C.

j. During the incubation, dilute the Anti-phosphotyrosine-Cy5 conjugate 1:1000 (6 µL plus 6 mL of Anti-phosphotyrosine Probing Buffer).

k. After incubation, carefully remove each array from the incubation dish with a pair of forceps and place in a clean Pap jar containing 25 mL of Functional Assay Buffer+DTT. Cap the tube, then **invert** several times. Place the tube on a shaker and shake gently at room temperature for 5 min to remove unbound probe. Pour off the Functional Assay Buffer+DTT.

l. Repeat the wash once more, **inverting** the container several times at each wash step.

m. Remove the array from the Functional Assay Buffer+DTT and drain the slide by blotting the long edge carefully onto lint-free tissue paper for 10 seconds.

n. Dry the back of the slide with lint-free tissue paper.

o. Place the slide into a chamber of a quadriPERM culture dish, as before. Ensure the slide does not rest on the plastic lugs at the numbered end of the chamber as this may lead to incomplete coverage of the slide. Immediately pipette 5 mL of the diluted Anti-phosphotyrosine-Cy5 conjugate from step j onto the slide, while minimizing introduction of bubbles. Add any additional slides to the chambers in the same way and replace the lid.

p. Place the chamber on an orbital shaker at room temperature and ensure the assay solution covers the entire array. Cover with foil to protect from light and shake dish gently for 30 min at room temperature.

q. After incubation, carefully remove each array from the incubation chamber with a pair of forceps and place in a clean Pap jar containing 25 mL of Functional Assay Buffer+DTT. Cap the tube, then **invert** several times. Shake gently for 5 min to remove unbound probe. Pour off the Functional Assay Buffer+DTT.

r. Perform two more 5 min washes, **inverting** the container several times at each wash step.

s. Pour off the final Functional Assay Buffer-DTT and add 25 mL of high purity water to wash away glycerol. Replace the lid on the container and **invert** several times before pouring off the water.

t. Immediately transfer the arrays to a 50 mL disposable centrifuge tube with the slide label at the bottom of the tube, using extreme care only to touch the slide label or edges to prevent damage to the array. Centrifuge the arrays at room temperature for 2 min at 240 $\times$ *g*.

u. Carefully remove the slides from the centrifuge tubes with a pair of dry, blunt-ended forceps, touching only the extreme end of the slide.

v. Scan the slides from the non-labeled end using a microarray scanner or imager.

Protocol for the Anti-c-Myc-Cy3 Binding Assay

After performing the functional assay, use the anti-c-Myc-Cy3 conjugate to demonstrate the level of protein present in each kinase protein feature.

a. Allow the Assay Buffer to equilibrate to room temperature. Perform all steps at room temperature. In order to perform the assay, it is necessary to make two buffers from the Assay Buffer.

b. Prepare the **Anti-c-Myc Wash Buffer+DTT**: add 150 µL of 1 M DTT to 150 mL of Assay Buffer immediately before use.

c. Prepare the **Anti-c-Myc Probing Buffer**: add 200 mg of BSA to 10 mL of Anti-c-Myc Wash Buffer+DTT from step b.

d. Dilute the anti-c-Myc-Cy3 conjugate1:1000 (5 µL plus 5 mL of anti-c-Myc Probing Buffer+DTT.)

e. Place slides in a clean Pap jar with 25 mL of Anti-c-Myc Wash Buffer+DTT and incubate with gentle shaking (40–50 rpm) for 5 min.

f. Remove each array with a pair of blunt-ended forceps. Drain excess liquid from the slide surface by resting the long edge of the slide on lint-free tissue paper for 10 seconds.

g. Dry the back of the slide on lint-free tissue paper.

h. Place the slide into a chamber of a quadriPERM culture dish so it is level horizontally with the array side facing up, then immediately pipette 2 mL of the diluted anti-c-Myc-Cy3 carefully onto the slide without introduction of bubbles. Add additional slides to the chambers in the same manner, then replace the lid. Ensure the slide does not rest on the plastic lugs at the numbered end of the chamber as this may lead to incomplete coverage of the slide. To protect the fluorophore, cover the quadriPERM vessel with foil.

i. Incubate the quadriPERM vessel at room temperature and shake gently for 6 hours. Most c-Myc positive proteins on the array can be visualized after a 6-hour incubation. To increase assay sensitivity, the incubation can be extended overnight up to 20 hours before processing. For longer incubations, place the quadriPERM dish in a sealed plastic container with a wad of damp tissues to prevent drying out.

j. After incubation, carefully remove each array from the incubation chamber with a pair of forceps and place in a clean Pap jar with 25 mL of Anti-c-Myc Wash Buffer+DTT (two arrays per tube). Cap the tube, then **invert** several times. Shake the tube gently for 5 min to remove unbound antibody. Pour off the Anti-c-Myc Wash Buffer+DTT.

k. Repeat two more times, **inverting** the container several times at each wash step.

l. Pour off the final Anti-c-Myc Wash Buffer+DTT and add 25 mL of water to the Pap jar. Cap the tube and **invert** several times to remove glycerol from the slide surface.

m. Using forceps, place each slide in a 50 mL disposable centrifuge tube with the label at the bottom of the tube. Be careful only to handle slides by the label or by the edges of the slide to avoid damaging the array. Centrifuge the arrays at room temperature for 2 min at 240 × *g*.

n. Carefully remove the slides from the centrifuge tubes with a pair of dry forceps, touching only the extreme end of the slide.

o. Scan the slides from the non-labeled end first using a microarray scanner or imager.

Scanning

a. Protein function arrays can be scanned using conventional microarray scanners and imagers to detect fluorescence.

b. Scanning should be performed according to the manufacturer's recommended protocols. Care should be taken not to scratch the array surface during handling.

c. Excitation and emission wavelengths for Cy5 are λ_{ex} 649 nm and λ_{em} 670 nm, respectively. Cy3 excitation and emission are λ_{ex} 550 nm and λ_{em} 570 nm, respectively.

d. It is advisable to perform a pre-scan to adjust the scanning parameters and obtain an optimal image. Final images should have the following properties: the most intense spots should not be saturated and background should be as low as possible. If the array image is faint, repeat the scanning with increased Laser Power (%) and/or gain (PMT voltage) as necessary.

   In the opposite case, repeat the scanning process at lower Laser Power (%) and/or lower PMT settings. Scanning should be repeated to obtain an optimal image, bearing in mind that "overexposure" can lead to photobleaching of fluorophores.

e. Images should be saved as TIFF files for future analysis.

Data Analysis

Analyze the images using a standard microarray analysis software package. After loading the TIFF file, analyze the array with an array-specific grid using the GAL file provided with the kit. The GAL file will also provide annotation for each spot on the array. Once the GAL file is loaded, adjust the grid so the marker spots are aligned in the top left corner of each sub-grid. This may be followed by auto-alignment. After auto-alignment, it may be necessary to manually edit individual subarrays to ensure optimal alignment. Follow the guidelines of the software manufacturer. For optimal results, use local background subtraction and measure net signal intensities using median pixel intensity values. Set the spot size to 400 µm.

When the grid alignment is complete, export the data in the order determined by the GAL file to the Excel spreadsheet provided with the kit. Cut and paste the column of data for each slide into the blue columns in the Cy3 or Cy5 "Input" sheets of the Panorama Kinase Array v1 Analysis Workbook. The Excel spreadsheet automatically sorts the data and determines the mean and standard deviation of the four replicates for each protein feature on the array. If required, sort the processed data manually according to the spot intensity and display the data graphically using standard Excel procedures.

Data Normalization

The amount of material for each protein on the array can vary depending on expression levels. Take the variation of protein amount into consideration when analyzing data from microarray experiments. The Kinase v1 array includes biotin-BSA to detect any general non-specific binding. Other controls include proteins with and without the c-Myc epitope tag (β-galactosidase-BCCP-Myc, β-galactosidase-BCCP, BCCP-Myc and BCCP), which will indicate any non-specific interactions related to the folding and detection tags.

The controls measure any non-specific interactions and set a background level against which to determine significant interactions. Perform the anti-c-Myc-Cy3 assay on each slide after the primary assay to measure the amount of protein in every spot. The data obtained from the anti-c-Myc-Cy3 assay can be used to normalize the data from other assays performed on the array.

Standard Normalization

The signal from the primary assay is divided by the signal from the anti-c-Myc-Cy3 assay, thereby allowing normalization of the assay result to the amount of protein in each spot. After determining the mean and standard deviation for each of the four replicates for each protein, plot the data.

Note: If values for the anti-c-Myc-Cy3 conjugate assay are particularly low, do not use the values for normalization as this may lead to unreliable normalization of the primary assay data. As a guide, do not normalize values in the anti-c-Myc-Cy3 conjugate assay that are not significantly greater than the binding to control proteins using this method. Detection of individual proteins using the anti-c-Myc-Cy3 assay can be affected by the scanning parameters used, prior functional assays on the array, and occlusion of the c-Myc epitope.

As with any kind of normalization, consider whether normalization has introduced any misleading results. The following criteria should be used to corroborate positive results:

- Check magnitude of non-normalized data in both assays to ensure the normalized data is realistic.
- Ensure data for each of the four replicate spots display the same trend.
- Visually check positives on the original array images.

As with any array technology, validate any positive data spots by independent techniques such as immunoprecipitation assays or electrophoretic mobility shift.

Guidelines for Different Assay Types

General Recommendations

Design buffers in accordance with known literature for the particular assay under investigation, taking due consideration of the need for particular ions or cofactors. Where suitable, it is recommended that all buffers include 20% glycerol, 1 mM DTT, 0.1% Triton X-100, and at least 0.1% BSA to stabilize proteins on the array.

Kinase v1 arrays have been blocked with 2% BSA; however, depending on the assay, it may be necessary to block further. To minimize non-specific binding, perform protein interaction and antibody binding assays in the presence of blocking materials such as 2% BSA or dried low-fat milk powder. Blocking with 5% milk powder is not recommended for work involving phosphorylation as it contains high concentrations of phosphoproteins that can increase background. If biotinylated probes are to be used, block the streptavidin surface with buffers including 20 µM biotin. In such cases, it is advisable to probe in buffers containing 5% dried low-fat milk powder.

When labeling proteins or peptides with fluorescent dyes, do not exceed a labeling ratio of 1–2 dye moieties per molecule to ensure there is minimal interference with binding.

Label peptide and oligonucleotide probes during synthesis and purify them via HPLC. An aminohexanoic acid spacer between the fluorophore and peptide is appropriate for short sequences in which steric hindrance may be problematic.

Store labeled probes (e.g., DNA, peptides, proteins, or antibodies) in aliquots at –20 °C. Do not freeze/thaw aliquots more than once.

Where applicable, perform assays at 4 °C to maximize protein stability.

Phosphorylation on Arrays Using Exogenous Kinases

To determine which proteins are potential substrates for particular kinases, the proteins on the Kinase v1 array can be used as substrates for exogenous kinases.

Use the protocol for the Autophosphorylation Assay described in this guide as the basis for development of assays using exogenous kinases. Refer to General Recommendations for additional information.

Phosphorylation events can be successfully detected using labeled anti-phosphotyrosine, anti-phosphoserine, anti-phosphothreonine, or radiolabelled ATP.

Add exogenous kinases to the probing solution at a concentration in the region of 10 nM.

Perform phosphorylation reactions at 20–30 °C.

Assays Utilizing Radioactive ATP

Phosphorylation of kinases on the Kinase v1 array can be detected using radiolabeled ATP. To perform phosphorylation using radiolabeled ATP, follow the general procedure described for the Autophosphorylation Assay.

Ensure the final concentration of ATP exceeds 100 µM and has a specific activity of >60 Ci/mmol [γ-^{33}P]-ATP.

Exogenous kinases can be added to the reaction at a concentration in the range of 10 nm.

To reduce the amount of radioactivity used, perform incubations in low volumes. Perform assays using 50 µL of probing solution under a HybriSlip supplied with this kit.

Exogenous kinases may autophosphorylate in the presence of ATP. It is important to ensure the kinase is thoroughly removed from the array before detection. To reduce non-specific binding of the exogenous

kinase or radiolabeled probe to the array surface, wash the arrays once with water, followed by two washes with 0.5% SDS in water, then wash the arrays twice in high purity water to remove the detergent before drying and detection. Perform each wash for 5 min with shaking at room temperature.

Phosphorylation can be imaged using autoradiography or phosphorimaging.

DNA Binding Assays

It is recommended that oligonucleotides are 5'-labeled with fluorophores during synthesis (available from Sigma-Genosys). Both strands of double-stranded DNA probes can be labeled to maximize sensitivity.

When selecting probes, it is recommended to apply the same considerations as for any other DNA: protein study. Probes should be of the highest purity possible to generate unequivocal results. Oligonucleotides should not include repeat or lengthy sequences that may give rise to secondary structures.

Store aliquots of oligonucleotides at –20 °C. Do not freeze/thaw aliquots of probe more than once.

Antibody Binding Assays

Antibody labeling may be performed using mono-reactive Cy-dyes.

Desalt labeled probes to remove free dye before use.

Some dye labels lead to higher levels of non-specific binding than others. If high non-specific binding is observed, it may be necessary to evaluate other dyes.

When preparing labeled antibodies, prepare aliquots and store at –20 °C. Do not freeze/thaw aliquots of antibodies more than once.

Antibody binding can be detected by direct labelling of the antibody or by secondary detection using another antibody or probe. To simplify assay development, it is recommended to use direct detection where applicable.

Protein:Protein Interactions

The optimal conditions for studying protein:protein interactions on the array will vary according to the protein being studied.

The optimal concentration of protein probe will depend on the affinity of interaction with arrayed proteins. Choose the probe concentration in accordance with the known literature or experience.

Protein:protein interactions can be detected by direct labeling or by indirect detection using labeled antibodies or probes. To simplify assay development, it is recommended to use direct detection where applicable.

Post-translational Modification

Protein function arrays can be used to determine whether proteins on the array are potential substrates for specific enzymes such as kinases. These arrays can be used to determine the effect of post-translational modification *in vitro* on protein function.

The proteins on the array can be modified enzymatically on the array and detected using labeled antibodies specific for a particular modification.

Troubleshooting Guide

Signal is very low over the entire array

- Fluorescent labeling of the molecule used as probe may not have been efficient. Check the degree of labeling of the probe (1–2 molecules of dye per protein) and either repeat the labeling or use a higher amount of probe.
- Assay conditions used may be sub-optimal for any new interaction or reaction under study. Optimize the conditions as appropriate including blocking conditions, buffer composition, assay incubation time, and temperature.
- Check literature for specific conditions required for interaction of the probe with particular protein targets.

Signal for replicate spots is inconsistent

- When using cover slips, ensure that no air bubbles are trapped during assay.
- When assaying in larger volumes, ensure the arrays are completely covered with liquid and not allowed to dry out during assay.
- When using quadriPERM dishes, ensure the slide does not rest on the plastic lugs at the numbered end of the chamber.

Streaking is observed on the array after scanning

- This may occur when slides are dried by centrifugation and the array label is placed at the top. Always dry the slides with the label at the bottom of the tube.

Spots on the array appear scratched

- If using cover slips, be careful not to drag the cover slip across the array surface when placing or removing.
- When using forceps, be certain to grasp the slide at either end and be certain not to touch the area where proteins are printed.

The background is speckled

- Use clean powder-free gloves when handling the arrays.
- Ensure assay containers are clean and free of fluorescent contaminants.
- Where applicable, ensure buffers are prepared freshly before use.
- Apply despeckling algorithms present in most commercially available microarray analysis software packages.

  Note: The speckling phenomenon does not materially affect the data generated.

The background signal is high over the entire surface

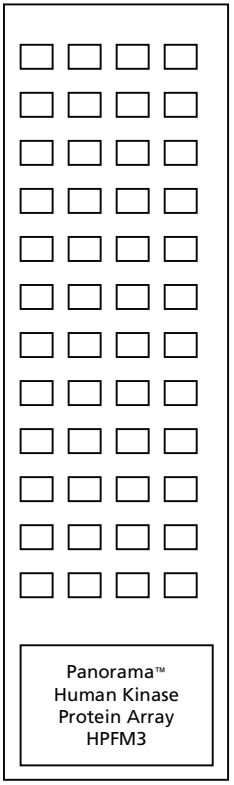
- Re-scan at lower laser power and/or PMT setting.
- Further blocking before and/or during the assay may be required to prevent non-specific interactions. Blocking is assay and sample dependant. Try using commonly used blocking agents (i.e., non-fat milk or increased concentrations of BSA).
- When using a labeled antibody probe, the concentration of the probe must be optimized to minimize non-specific binding.

There are localized patches of high background on the slides

- Ensure arrays are completely covered with buffer during wash steps.
- Ensure arrays are not allowed to dry out during assay or processing.

Appendices

Appendix A: Protein Array Orientation



The Human Kinase Array consists of forty-eight, 5 × 4 subgrids. Two Cy3/Cy5 biotin BSA marker spots are situated in the top left corner of the first and fourth sub-arrays of each row of subpanels. A dilution series of control proteins is situated in the top line of the second column of sub-arrays.

Appendix B: Panorama Human Protein Function Array Kinase v1—Protein Identities

Symbol	Gene Name	Entrez Gene ID
AAK1	AP2 associated kinase 1	22848
ACVR1	activin A receptor, type I	90
ACVR1C	activin A receptor, type IC	130399
ADCK4	aarF domain containing kinase 4	79934
ADRBK2	adrenergic, beta, receptor kinase 2	157
AKT1	v-akt murine thymoma viral oncogene homolog 1	207
ALS2CR2	amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 2	55437
APEG1	aortic preferentially expressed protein 1	10290
ARAF1	v-raf murine sarcoma 3611 viral oncogene homolog 1	369
AURKB	serine/threonine kinase 12	9212
BLK	B lymphoid tyrosine kinase	640
BMX	BMX non-receptor tyrosine kinase	660
BRD3	bromodomain containing protein 3	8019
BUB1	BUB1 budding uninhibited by benzimidazoles 1 homolog (yeast)	699
BUB1B	BUB1 budding uninhibited by benzimidazoles 1 homolog beta (yeast)	701
C20orf64 / PRPK	chromosome 20 open reading frame 64	112858
C20orf97	chromosome 20 open reading frame 97	57761
CAMK1G	calcium/calmodulin-dependent protein kinase IG	57172
CAMK2B	calcium/calmodulin-dependent protein kinase (CaM kinase) II beta	816
CAMK2D	calcium/calmodulin-dependent protein kinase (CaM kinase) II delta	817
CAMK2G	calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma	818
CAMK4	calcium/calmodulin-dependent protein kinase IV	814
CAMKK1	calcium/calmodulin-dependent protein kinase kinase 1, alpha	84254
CAMKK2	calcium/calmodulin-dependent protein kinase kinase 2, beta	10645
CCRK	cell cycle related kinase	23552
CDC2	cell division cycle 2, G1 to S and G2 to M	983
CDK2	cyclin-dependent kinase 2	1017
CDK4	cyclin-dependent kinase 4	1019
CDK5	cyclin-dependent kinase 5	1020
CDK7	cyclin-dependent kinase 7	1022
CDK9	cyclin-dependent kinase 9	1025
CDKN2A	cyclin-dependent kinase inhibitor 2A	1029
CHEK1	CHK1 checkpoint homolog *(S. pombe)*	1111

Symbol	Gene Name	Entrez Gene ID
CHEK2	CHK2 checkpoint homolog *(S. pombe)*	11200
CLK1	CDC-like kinase 1	1195
CLK2	CDC-like kinase 2	1196
CLK3	CDC-like kinase 3	1198
COL4A3BP	Similar to collagen, type IV, alpha 3 (Goodpasture antigen) binding protein	10087
CSNK1D	casein kinase 1, delta	1453
CSNK1G1	casein kinase 1, gamma 1	53944
CSNK1G2	casein kinase 1, gamma 2	1455
CSNK2A1	casein kinase 2, alpha 1 polypeptide	1457
CSNK2A2	casein kinase 2, alpha prime polypeptide	1459
DYRK2	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2	8445
DYRK4	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 4	8798
FASTK	FAST kinase	10922
FES	feline sarcoma oncogene	2242
FGFR2	fibroblast growth factor receptor 2	2263
FLJ14813	hypothetical protein FLJ14813	84930
FLJ20574	hypothetical protein FLJ20574	54986
FRK	fyn-related kinase	2444
FYN	FYN oncogene related to SRC, FGR, YES	2534
GPRK6	G protein-coupled receptor kinase 6	2870
GSK3B	glycogen synthase kinase 3 beta	2932
H11	protein kinase H11	26353
HK1	hexokinase 1	3098
IKBKB	inhibitor of kappa light polypeptide gene enhancer in B-cells kinase beta	3551
ILK	integrin-linked kinase	3611
JIK	STE20-like kinase	51347
KIS	kinase interacting with leukemia-associated gene (stathmin)	127933
LCK	lymphocyte-specific protein tyrosine kinase	3932
LIMK2	LIM domain kinase 2	3985
MAK	male germ cell-associated kinase	4117
MAP2K3	mitogen-activated protein kinase kinase 3	5606
MAP2K5	mitogen-activated protein kinase kinase 5	5607
MAP2K6	mitogen-activated protein kinase kinase 6	5608
MAP2K7	mitogen-activated protein kinase kinase 7	5609
MAP3K14	mitogen-activated protein kinase kinase kinase 14	9020
MAP3K6	mitogen-activated protein kinase kinase kinase 6	9064
MAP3K7	mitogen-activated protein kinase kinase kinase 7	6885
MAP4K5	mitogen-activated protein kinase kinase kinase kinase 5	11183

Symbol	Gene Name	Entrez Gene ID
MAPK1	mitogen-activated protein kinase 1	5594
MAPK11	mitogen-activated protein kinase 11	5600
MAPK13	mitogen-activated protein kinase 13	5603
MAPK14	mitogen-activated protein kinase 14	1432
MAPK3	mitogen-activated protein kinase 3	5595
MAPK6	mitogen-activated protein kinase 6	5597
MAPK7	mitogen-activated protein kinase 7	5598
MAPK9	mitogen-activated protein kinase 9	5601
MAPKAPK3	mitogen-activated protein kinase-activated protein kinase 3	7867
MARK3	MAP/microtubule affinity-regulating kinase 3	4140
MATK	megakaryocyte-associated tyrosine kinase	4145
MGC16169	hypothetical protein MGC16169	93627
MGC42105	hypothetical protein MGC42105	167359
MGC45428	hypothetical protein MGC45428	166614
MKNK1	MAP kinase-interacting serine/threonine kinase 1	8569
NEK11	NIMA (never in mitosis gene a)- related kinase 11	79858
NEK3	NIMA (never in mitosis gene a)-related kinase 3	4752
PACE-1	ezrin-binding partner PACE-1	57147
PAK4	p21(CDKN1A)-activated kinase 4	10298
PCK2	phosphoenolpyruvate carboxykinase 2 (mitochondrial)	5106
PCTK1	PCTAIRE protein kinase 1, transcript variant 2	5127
PCTK1	PCTAIRE protein kinase 1, transcript variant 3	5127
PCTK2	PCTAIRE protein kinase 2	5128
PDK1	pyruvate dehydrogenase kinase, isoenzyme 1	5163
PDK2	pyruvate dehydrogenase kinase, isoenzyme 2	5164
PDK3	pyruvate dehydrogenase kinase, isoenzyme 3	5165
PDK4	pyruvate dehydrogenase kinase, isoenzyme 4	5166
PHKG2	phosphorylase kinase, gamma 2 (testis)	5261
PIK3C3	phosphoinositide-3-kinase, class 3	5289
PIK3R1	phosphoinositide-3-kinase, regulatory subunit, polypeptide 1 (p85 alpha)	5295
PIM1	pim-1 oncogene	5292
PIM2	pim-2 oncogene	11040
PKE	PKE protein kinase	282974
PKLR	pyruvate kinase, liver and RBC	5313
PKM2	pyruvate kinase, muscle	5315
PLK	polo (Drosophia)-like kinase	5347

Symbol	Gene Name	Entrez Gene ID
PRKACB	protein kinase, cAMP-dependent, catalytic, beta	5567
PRKACG	protein kinase, cAMP-dependent, catalytic, gamma	5568
PRKCB1	protein kinase C, beta 1	5579
PRKCH	protein kinase C, eta	5583
PRKCI	protein kinase C, iota	5584
PRKCN	protein kinase C, nu	23683
PRKCZ	protein kinase C, zeta	5590
PRKD2	protein kinase D2	25865
PTK2	PTK2 protein tyrosine kinase 2	5747
PTK9	PTK9 protein tyrosine kinase 9	5756
PXK	PX domain containing serine/threonine kinase	54899
RAF1	v-raf-1 murine leukemia viral oncogene homolog 1	5894
RIOK2	RIO kinase 2 (yeast)	55781
RIOK3	RIO kinase 3 (yeast)	8780
RIPK2	receptor-interacting serine-threonine kinase 2	8767
RPS6KA1	ribosomal protein S6 kinase, 90kDa, polypeptide 1	6195
RPS6KA2	ribosomal protein S6 kinase, 90kDa, polypeptide 2	6196
RPS6KA5	ribosomal protein S6 kinase, 90kDa, polypeptide 5	9252
RPS6KL1	ribosomal protein S6 kinase-like 1	83694
SGK	serum/glucocorticoid regulated kinase	6446
SGKL	serum/glucocorticoid regulated kinase-like	23678
SNARK	likely ortholog of rat SNF1/AMP-activated protein kinase	81788
SNK	serum-inducible kinase	10769
SRC	v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian)	6714
SRPK1	SFRS protein kinase 1	6732
STK11	serine/threonine kinase 11 (Peutz-Jeghers syndrome)	6794
STK16	serine/threonine kinase 16	8576
STK17B	serine/threonine kinase 17b (apoptosis-inducing)	9262
STK22C	serine/threonine kinase 22C (spermiogenesis associated)	81629
STK22D	serine/threonine kinase 22D (spermiogenesis associated)	83942
STK24	serine/threonine kinase 24 (STE20 homolog, yeast)	8428
STK25	serine/threonine kinase 25 (STE20 homolog, yeast)	10494
STK3	serine/threonine kinase 3 (STE20 homolog, yeast)	6788
STK33	serine/threonine kinase 33	65975
STK38	serine/threonine kinase 38	11329
STK38L	serine/threonine kinase 38 like	23012
STK6	serine/threonine kinase 6	6790

Symbol	Gene Name	Entrez Gene ID
SYK	spleen tyrosine kinase	6850
TBK1	TANK-binding kinase 1	29110
TEK	TEK tyrosine kinase, endothelial	7010
TLK1	tousled-like kinase 1	9874
TTK	TTK protein kinase	7272
VRK3	vaccinia related kinase 3	51231
ZAK	sterile alpha motif and leucine zipper containing kinase AZK	51776
ZAP70	zeta-chain (TCR) associated protein kinase 70kDa	7535

Control and Marker Proteins

Protein Content Code	Symbol	Identify	Gene ID
Marker		Cy3/Cy5 labeled biotin-BSA marker proteins	
Control 1		β-gal-BCCP-Myc	
Control 2		BCCP-Myc	
Control 3		β-gal-BCCP	
Control 4		BCCP	
Controls 5–12		Insect Lysis Buffer	

Dilution Series

Features BCCP1 to BCCP6 represent a two-fold dilution series of BCCP-Myc starting from the standard lysate concentration used for all other recombinant proteins.

Features BSA1 to BSA 6 represent a two-fold dilution series of biotin-BSA starting at a spotting concentration of 25 ng/µL.

Biotin BSA Standard	Spotting Concentration
BSA1	25 ng/µL
BSA2	12.5 ng/µL
BSA3	6.25 ng/µL
BSA4	3.125 ng/µL
BSA5	1.56 ng/µL
BSA6	0.78 ng/µL

Features NP1 to NP6 represent a two-fold dilution series of a non-phosphorylated peptide (biotin-Ahx-KVEKIGEGTYGVVKK-CONH2) which acts as a substrate for certain kinases such as FES kinase.

Non-phosphorylated Peptide Standard	Spotting Concentration
NP1	156 pg/µL
NP2	78 pg/µL
NP3	39 pg/µL
NP4	19.5 pg/µL
NP5	9.8 pg/µL
NP6	4.9 pg/µL

Features PP1 to PP6 represent a two-fold dilution series of a phosphorylated peptide (biotin-Ahx-KVEKIGEGT[pY]GVVKK-CONH2) which is recognized by anti-phosphotyrosine Cy5 supplied with this kit.

Phosphorylated Peptide Standard	Spotting Concentration
PP1	156 pg/µL
PP2	78 pg/µL
PP3	39 pg/µL
PP4	19.5 pg/µL
PP5	9.8 pg/µL
PP6	4.9 pg/µL

References

1. A census of human cancer genes (2004) *Nature Reviews Cancer,* **4**, 177-183, Futreal PA, Coin L, Marshall M, Down T, Hubbard T, Wooster R, Rahman N and Stratton MR.
2. Functional protein microarrays for parallel characterisation of p53 mutants (2004) *Proteomics*, **4**, 1950-1958, Boutell JM, Hart DJ, Godber BLJG, Kozlowski RZ and Blackburn JM.
3. Strategies for immobilisation of biomolecules in a microarray (2004), *Combinatorial Chemistry & High Throughput Screening*, **7**, 213-221, Yeo DSY, Panicker RC, Tan L-P and Yea SQ.
4. Enzymatic activity on a chip: The critical role of protein orientation (2005) *Proteomics*, **5**, 416-419, Cha TW, Guo A and Zhu X-Y.
5. Development of Protein Microarrays for Drug Discovery (2004) Koopmann JO, McAndrew MB and Blackburn JM in Protein Microarrays; Mark Schena (Ed), pp 401-420.
6. Reversible, site specific immobilization of polyarginine-tagged fusion proteins on mica surfaces. (1997) *FEBS Letters* **414**, 233-238, Nock S., Spudich, J.A., Wagner, P.

Relevant Patents

1. WO 01/57198. Methods Of Generating Protein Expression Arrays And The Use Thereof In Rapid Screening.
2. WO 02/27327. Rapid Profiling Of The Interactions Between A Chemical Entity And Proteins In A Given Proteome.
3. WO 03/048768. Protein Arrays For Allelic Variants And Uses Thereof.
4. WO 03/064656. Protein Tag Comprising A Biotinylation Domain and Method for Increasing Solubility and Determining Folding State.

Notes

Notes

World Headquarters
3050 Spruce St., St. Louis, MO 63103
(314) 771-5765
sigma-aldrich.com

Order/Customer Service (800) 325-3010 • Fax (800) 325-5052
Technical Service (800) 325-5832 • sigma-aldrich.com/techservice
Development/Bulk Manufacturing Inquiries SAFC (800) 244-1173

The SIGMA-ALDRICH Group

SIGMA · ALDRICH · Fluka · Riedel-de Haën · SAFC · SUPELCO · ISOTEC

SIGMA-ALDRICH

*Accelerating Customers' Success through Leadership in **Life Science**, **High Technology** and **Service***

00334-502906
0036