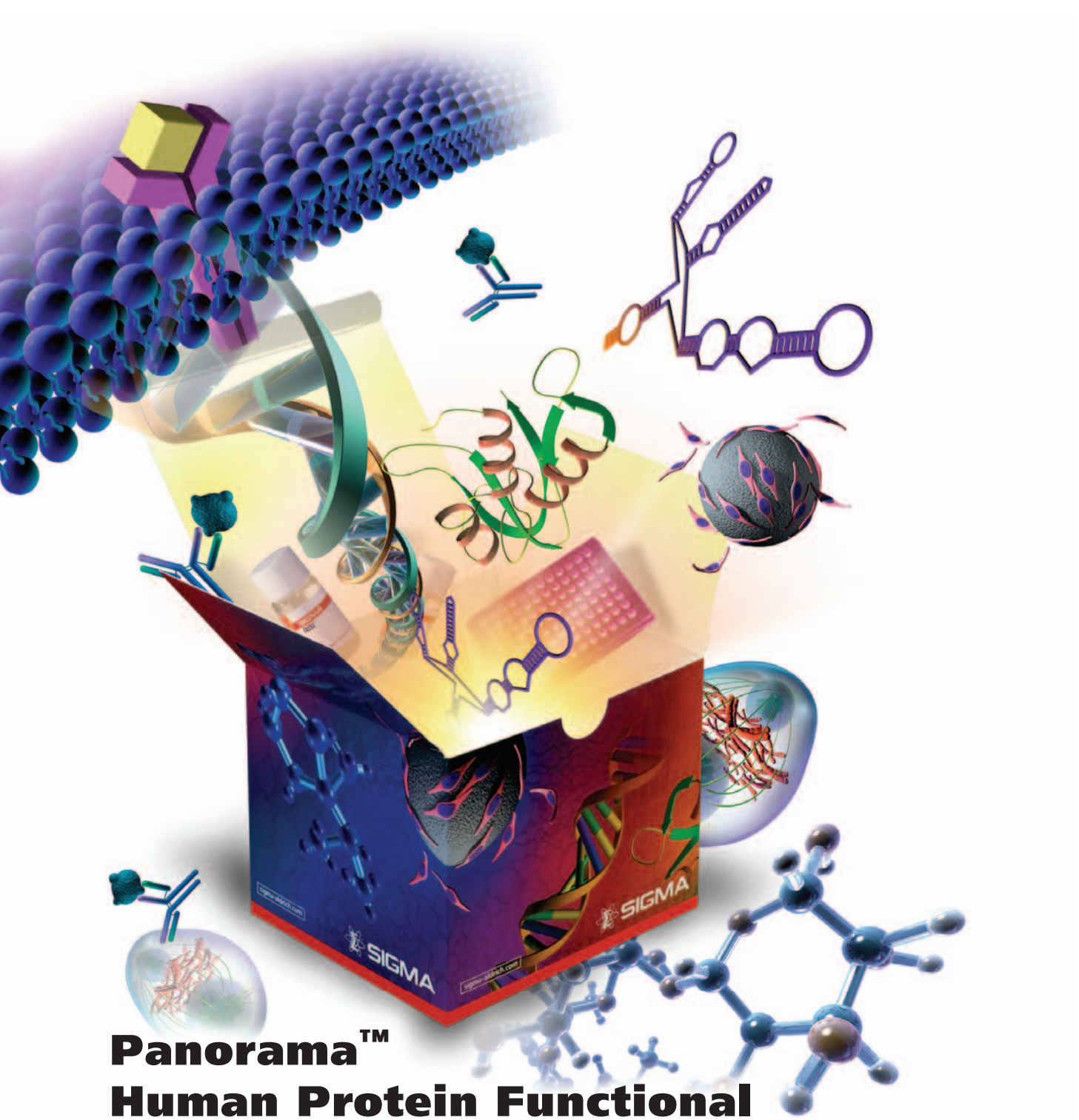


# Panorama™ Human Protein Functional Array-Signal Transduction

# Technical Bulletin

Catalog Number
**HPFM4**

SIGMA-ALDRICH

# Panorama™ Signal Transduction Array

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# Introduction

The Panorama™ Signal Transduction Array contains a set of 259 human signaling proteins and specific controls. These proteins are intimately involved in many cell processes such as cell proliferation, cell differentiation and cell death.

All proteins on array have been chosen because of their involvement in signaling networks. The majority of proteins were selected using GO-terms according to the Gene Ontology database.[1]

# Panorama Array Technology

The signal transduction proteins were expressed in S*f*9 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 signal transduction proteins 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 and the c-Myc epitope (EQKLISEEDL), which can be used to visualize the proteins on the array. To ensure fidelity, clones are sequence-verified immediately prior to expression in S*f*9 insect cells.

During expression, the BCCP tag is biotinylated only when it is correctly folded.[5] All expressed proteins are assayed by Western blot analysis to determine molecular weight, confirm biotinylation, and establish the 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 Signal Transduction Array | **P4249** | 2 each |
| BCR-peptide-Cy5 | **B2936** | 1 each |
| Anti-c-Myc-Cy3 | **C6594** | 1 each |
| Functional Assay Buffer | **C0492** | 150 mL |
| Assay Buffer | **A1105** | 150 mL |
| CHAPS | **C3023** | 1 g |
| Bovine Serum Albumin (BSA) | **A3059** | 1 × 200 mg |
| 1M Dithiothreitol (DTT) | **646563** | 2 ampoules |
| quadriPERM® culture vessel | **Z376760** | 2 each |

| Product | Cat. No. | Size |
|---|---|---|
| Hybri-slips™ | **H0784** | 10 each |
| Pap Jar | **P8123** | 4 each |
| 50 mL Conical Centrifuge Tubes | **C8296** | 2 each |
| Signal Transduction Analysis Workbook and GAL File (CD) | **S4571** | 1 each |

### Materials Required But Not Provided

The following materials are not included in the protein array kit, but are required to perform the peptide and antibody binding assays described in this technical bulletin.

- 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 DTT 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. Arrays must not be moved during thawing. Pap jars should not be opened until the storage buffer has thawed completely. It is recommended that tubes are not opened until ready for 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. 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 Hybri-slips 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. During assay cover dishes with aluminum foil.

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

h. Signal Transduction 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 de-speckling algorithms that are present in most commercially available microarray analysis software. Please note that speckling affects the appearance only and does not materially affect the data generated.

# Kit Components

## Protein Arrays

Each Signal Transduction array contains 259 wild type signal transduction proteins, numerous control features, and 48 alignment markers in 48 sub-grids per slide. The signal transduction proteins within the arrays have been printed in quadruplicate in 6 × 5 subarrays. Each feature on the array has a spot diameter of approximately 500 µm spaced at intervals of 300 µm. 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 list of the Signal Transduction proteins are shown in Appendices A and B.

Each Signal Transduction array contains:

- 259 human signal transduction 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 ß-galactosidase fused with the immobilization tag BCCP
- 4 anti-c-Myc positive control spots consisting of BCCP-c-Myc
- 4 anti-c-Myc positive control spots consisting of β-galactosidase fused with the immobilization tag 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 can act as a substrate for certain tyrosine kinases.
- Dilution series of negative control spots consisting of biotin-BSA
- 4 negative control spots printed with lysis buffer
- 4 negative control spots printed with spotting buffer

The proteins are arrayed onto a streptavidin-coated, low fluorescence glass slide (25 × 75.6 x 1 mm) that is compatible with conventional microarray instrumentation. The upper side and orientation of the array 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.

## BCR-peptide-Cy5

A peptide binding assay using a Cy5-labeled BCR peptide is supplied as a control assay to test the functionality of proteins on the Signal Transduction array. The sequence of the peptide is Cy5-C-Ahx-GSGSGKPF[pY]VNVEF-OH in which the motif KPF[pY]VNVEF corresponds to residues 174-182 of BCR_HUMAN, which binds specifically to the SH2 domain of GRB2_HUMAN (growth factor receptor-bound protein 2). Perform the peptide binding assay with the Functional Assay buffer provided with the kit. GRB2 is an important signaling molecule that interacts with certain tyrosine-phosphorylated proteins such as IRS1, SHC and LNK via its SH2 and SH3 domains. The assay demonstrates the utility of the Signal Transduction array to investigate peptide motif binding specificity in a parallel fashion.

## 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 signal transduction proteins 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 and for data normalization. Perform the anti-c-Myc binding assay with the Assay Buffer provided with the kit.

# Control Assays

## Protocol for Peptide Binding Assay

a. Allow the Functional Assay Buffer to equilibrate to room temperature. Perform all steps at room temperature.

b. Prior to performing the assay, add 150 mg of CHAPS and 150 µl of 1 M DTT to 150 mL of the Functional Assay Buffer. Dissolve and mix thoroughly.

c. Prepare the **Peptide Assay Probing Solution**: Dissolve the BCR-peptide-Cy5 in 25 µL of Functional Assay Buffer+CHAPS/DTT from step b and vortex thoroughly to completely dissolve. Centrifuge briefly to deposit the BCR-peptide solution in the bottom of the tube then pipette 22 µL of the solution into 5.5 mL of Functional Assay Buffer+CHAPS/DTT and mix thoroughly. This results in a 40 nM solution of BCR-peptide.

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

e. While the array is shaking, pipette 5 mL of the Peptide Assay Probing Solution from step c into one chamber of the quadriPERM culture dish.

f. Remove array from the Functional Assay Buffer+CHAPS/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 on the lint-free tissue paper.

h. Place the slide into the filled chamber of the quadriPERM culture dish so that it is horizontal with array side (label 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. Add any additional slides to the chambers in the same manner and then replace the lid.

i. Place the dish on a shaker at room temperature. Ensure the assay solution covers the entire array. Shake the dish gently (40–50 rpm) for 30 minutes at room temperature.

j. 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+CHAPS/DTT. Cap the tube then **invert** several times. Place the tube on a shaker and shake gently (40–50 rpm) for 5 minutes to remove unbound probe. Pour off the Functional Assay Buffer+CHAPS/DTT.

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

l. Pour off the final Functional Assay Buffer+CHAPS/DTT wash 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.

m. Immediately transfer the arrays to a 50 mL disposable centrifuge tube positioning the slide with the 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 minutes at 240 × *g*.

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

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

## Protocol for the Cy3-anti-c-Myc Binding Assay

After performing the functional assay, use the anti-c-Myc-Cy3 to demonstrate the level of protein present in each signal transduction protein feature. Perform all steps at room temperature.

a. Allow the Assay Buffer to equilibrate to 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).

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

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 horizontal with the array side (label side) facing up, then immediately pipette 2 mL of anti-c-Myc-Cy3 carefully onto the slide without introduction of bubbles. Add additional slides to the chambers in the same manner and 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 (40–50 rpm) 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 together 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 blunt-ended 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 minutes to remove unbound antibody. Pour off the Anti-c-Myc Wash Buffer+DTT.

k. Perform two more 5 minute washes, **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 high purity water to the Pap jar. Cap the tube and **invert** several times to remove glycerol from the slide.

m. Using blunt-ended 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 blunt-ended 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 $\lambda_{ex}$ 649 nm and $\lambda_{em}$ 670 nm, respectively. Cy3 excitation and emission wavelengths are $\lambda_{ex}$ 550 nm and $\lambda_{em}$ 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 lower PMT settings. Scanning should be repeated in order 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 Signal Transduction Array Analysis Workbook. The Excel spreadsheet automatically sorts the data and determines the mean and standard deviation for 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 levels of expression. Take the variation of protein amount into consideration when analyzing data from microarray experiments. The Signal Transduction 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 conjugate 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 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 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, electrophoretic mobility shift or solution assays.

## 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, include 20% glycerol, 1 mM DTT, 0.1% Triton X-100 and at least 0.1% BSA to stabilize proteins on the array.

Signal Transduction 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 may increase background. If biotinylated probes are to be used, block the streptavidin surface with buffers incorporating 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 that there is minimal interference with binding.

Label peptide and oligonucleotide probes during synthesis and purify them by HPLC.

Store labeled probes (e.g., DNA, peptides or proteins, including antibodies) in aliquots at –20°C. Do not freeze/thaw aliquots of probes 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 Signal Transduction Array can be used as substrates for exogenous kinases.

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 applicable, include 20% glycerol, 1 mM DTT, 0.1% Triton X-100 and at least 0.1% BSA in all buffers to stabilize proteins on the array.

Phosphorylation events can be detected successfully using labeled anti-phosphotyrosine and anti-phosphoserine or anti-phosphothreonine antibodies or by utilizing radiolabelled ATP.

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

Perform phosphorylation reactions at 20–30 °C.

## Assays Utilizing Radioactive ATP

Phosphorylation of proteins on these arrays can be detected using radiolabeled ATP.

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

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

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

Exogenous kinases may auto-phosphorylate 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 minutes with shaking at room temperature.

Phosphorylation can be detected 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. Oligos 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 labeling 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.

## Peptide Binding Assays

Peptide labeling may be performed using fluorescent compounds and this is best achieved during synthesis (available from Sigma-Genosys).

Peptides may be labeled at the N or C-terminus.

To circumvent steric hindrance, it is recommended that a spacer such as aminohexanoic acid is incorporated between the peptide and the labeling moiety.

Incorporation of a cysteine residue next to the spacer can act as a specific labeling moiety by use of maleimide reactive fluorophores provided no other cysteines are present in the sequence.

Peptides should be purified by HPLC to remove free dye and reaction contaminants after synthesis.

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.

To assay highly basic peptides, it is recommended to pre-block the arrays with 0.01% poly-L-lysine (Catalog Number P8920) in Functional Assay Buffer for 5 minutes at room temperature.

When preparing peptides, it is recommended to aliquot and dry the peptides with a small amount of carrier protein (e.g., 5–20 µg BSA per tube) to reduce losses and to maximize long-term stability. Dry the peptides using centrifugal evaporation. Once dissolved in buffer, it is recommended to use peptide solutions immediately.

## 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 the 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, assay buffer, assay incubation time and temperature.
- Check literature for specific conditions required for interaction of your 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 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 de-speckling 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

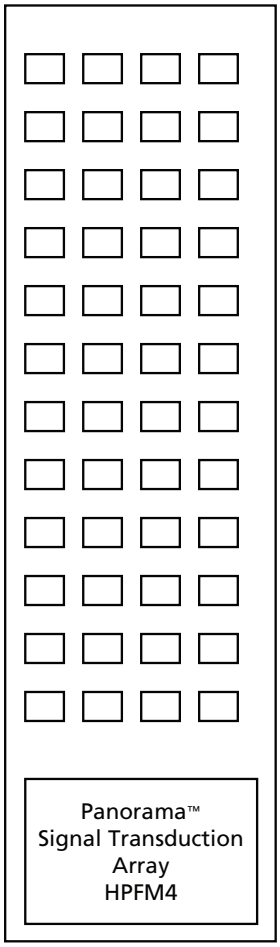
- Re-scan at lower laser power and PMT setting.
- Further blocking before and/or during the assay may be required to prevent non-specific interactions. Blocking is assay and sample dependant. Try using commonly used blocking agents (e.g., non-fat milk or increased concentrations of BSA.)
- When using 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 Signal Transduction Array consists of forty-eight, 6 × 5 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 Signal Transduction Array Content

| Symbol | Gene Name | Entrez Gene ID |
|---|---|---|
| ABI1 | Abl interactor 1 | 10006 |
| ACVR1 | activin A receptor, type I | 90 |
| ADD1 | adducin 1 (alpha) | 118 |
| ADRBK2 | adrenergic, beta, receptor kinase 2 | 157 |
| AKT1 | v-akt murine thymoma viral oncogene homolog 1 | 207 |
| AMD1 | adenosylmethionine decarboxylase 1 | 262 |
| ANXA1 | annexin A1 | 301 |
| APEX1 | APEX nuclease (multifunctional DNA repair enzyme) | 328 |

| Symbol | Gene Name | Entrez Gene ID |
|---|---|---|
| ARAF1 | v-raf murine sarcoma 3611 viral oncogene homolog 1 | 369 |
| ARHA | ras homolog gene family, member A | 387 |
| ARRB1 | arrestin, beta 1, transcript variant 1 | 408 |
| ATF3 | activating transcription factor 3 | 467 |
| ATF4 | activating transcription factor 4 (tax-responsive enhancer element B67) | 468 |
| BAD | BCL2-antagonist of cell death | 572 |
| BCL10 | B cell lymphoma/leukemia 10 | 8915 |
| BCL2A1 | BCL2-related protein A1 | 597 |
| BDNF | brain-derived neurotrophic factor, transcript variant 5 | 627 |
| BIRC2 | baculoviral IAP repeat-containing 2 | 329 |
| BIRC3 | baculoviral IAP repeat-containing protein 3 | 330 |
| BIRC5 | baculoviral IAP repeat-containing 5 (survivin) | 332 |
| BLK | B lymphoid tyrosine kinase | 640 |
| BMP7 | bone morphogenetic protein 7 (osteogenic protein 1) | 655 |
| BMPR1A | bone morphogenetic protein receptor, type IA | 657 |
| BMX | BMX non-receptor tyrosine kinase | 660 |
| BRD8 | bromodomain containing 8 | 10902 |
| BTRC | beta-transducin repeat containing, transcript variant 1 | 8945 |
| BUB1B | BUB1 budding uninhibited by benzimidazoles 1 homolog beta (yeast) | 701 |
| CA1 | carbonic anhydrase I | 759 |
| CALM1 | calmodulin 1 (phosphorylase kinase, delta) | 801 |
| CALM2 | calmodulin 2 (phosphorylase kinase, delta) | 805 |
| CALM3 | calmodulin 3 (phosphorylase kinase, delta) | 808 |
| CAMK2B | calcium/calmodulin-dependent protein kinase (CaM kinase) II beta | 816 |
| CAMK2G | calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma | 818 |
| CAMK4 | calcium/calmodulin-dependent protein kinase IV | 814 |
| CAMKK2 | calcium/calmodulin-dependent protein kinase kinase 2, beta | 10645 |
| CASP3 | caspase 3, apoptosis-related cysteine protease | 836 |
| CASP9 | caspase 9, apoptosis-related cysteine protease | 842 |
| CCNB1 | cyclin B1 | 891 |
| CCND1 | cyclin D1 (PRAD1: parathyroid adenomatosis 1) | 595 |
| CCND2 | cyclin D2 | 894 |
| CCNH | cyclin H | 902 |
| CDC2 | cell division cycle 2, G1 to S and G2 to M | 983 |
| CDC20 | CDC20 cell division cycle 20 homolog (S. cerevisiae) | 991 |
| CDC25A | cell division cycle 25A | 993 |
| CDC25B | cell division cycle 25B | 994 |

| Symbol | Gene Name | Entrez Gene ID |
|---|---|---|
| CDC25C | cell division cycle 25C | 995 |
| CDC42 | cell division cycle 42 (GTP binding protein, 25kDa) | 998 |
| CDKN1A | cyclin-dependent kinase inhibitor 1A (p21, Cip1) | 1026 |
| CDKN2B | cyclin-dependent kinase inhibitor 2B | 1030 |
| CDKN2C | cyclin-dependent kinase inhibitor 2C | 1031 |
| CDKN2D | cyclin-dependent kinase inhibitor 2D | 1032 |
| CGEF2 | cAMP-regulated guanine nucleotide exchange factor II | 11069 |
| CHN1 | chimerin (chimaerin) 1 | 1123 |
| CISH | cytokine inducible SH2-containing protein | 1154 |
| CNN1 | calponin 1, basic, smooth muscle | 1264 |
| CREB1 | cAMP responsive element binding protein 1 | 1385 |
| CSEN | calsenilin, presenilin binding protein, EF hand transcription factor | 30818 |
| CSNK1D | casein kinase 1 | 1453 |
| CSNK1G1 | BC017236 , casein kinase 1 | 53944 |
| CSNK1G2 | casein kinase 1 | 1455 |
| CSNK2A1 | casein kinase 2, alpha 1 polypeptide | 1457 |
| CSNK2A2 | casein kinase 2, alpha prime polypeptide | 1459 |
| CSTB | cystatin B (stefin B) | 1476 |
| CTNNB1 | beta-catenin | 1499 |
| DDB1 | damage-specific DNA binding protein 1 | 1642 |
| DDIT3 | DNA-damage-inducible | 1649 |
| DNAJB1 | DnaJ (Hsp40) homolog, subfamily B | 3337 |
| DR1 | down-regulator of transcription 1, TBP-binding (negative cofactor 2) | 1810 |
| DSCR1 | Down syndrome critical region gene 1 | 1827 |
| DUSP6 | dual specificity phosphatase 6 | 1848 |
| DVL2 | dishevelled, dsh homolog 2 (Drosophila) | 1856 |
| EEF1A1 | eukaryotic translation elongation factor 1 alpha 1 | 1915 |
| EFS | embryonal Fyn-associated substrate | 10278 |
| EGR2 | early growth response 2 (Krox-20 homolog, Drosophila) | 1959 |
| EIF4E | eukaryotic translation initiation factor 4E | 1977 |
| EIF4EBP1 | eukaryotic translation initiation factor 4E binding protein 1 | 1978 |
| ELK3 | ELK3, ETS-domain protein (SRF accessory protein 2) | 2004 |
| EMS1 | ems1 sequence (mammary tumor and squamous cell carcinoma-associated) | 2017 |
| ENO1 | enolase 1 | 2023 |
| ENO2 | enolase 2 | 2026 |
| EPS15 | epidermal growth factor receptor substrate 15 | 2060 |
| ERG | transcriptional regulator ERG | 2078 |

| Symbol | Gene Name | Entrez Gene ID |
|---|---|---|
| ESR2 | estrogen receptor 2 (ER beta) | 2100 |
| ETS2 | v-ets erythroblastosis virus E26 oncogene homolog 2 | 2114 |
| EXT1 | exostoses (multiple) 1 | 2131 |
| EXT2 | exostoses (multiple) 2 | 2132 |
| EZH2 | enhancer of zeste homolog 2 (Drosophila) | 2146 |
| FADD | Fas (TNFRSF6)-associated via death domain | 8772 |
| FEN1 | flap structure-specific endonuclease 1 | 2237 |
| FES | feline sarcoma oncogene | 2242 |
| FGF1 | fibroblast growth factor 1 (acidic) | 2246 |
| FGF13 | fibroblast growth factor 13 | 2258 |
| FOLH1 | folate hydrolase (prostate-specific membrane antigen) 1 | 2346 |
| FRK | fyn-related kinase | 2444 |
| FYN | FYN oncogene related to SRC, FGR, YES | 2534 |
| GADD45G | growth arrest and DNA-damage-inducible | 10912 |
| GATA3 | GATA binding protein 3 | 2625 |
| GCLC | glutamate-cysteine ligase, catalytic subunit | 2729 |
| GEM | GTP binding protein overexpressed in skeletal muscle | 2669 |
| GNA15 | guanine nucleotide binding protein (G protein), alpha 15 (Gq class) | 2769 |
| GNAI2 | guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2 | 2771 |
| GNAI3 | guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 3 | 2773 |
| GNAO1 | guanine nucleotide binding protein (G protein), alpha activating activity polypeptide O | 2775 |
| GNAZ | guanine nucleotide binding protein (G protein), alpha z polypeptide | 2781 |
| GNB1 | guanine nucleotide binding protein (G protein), beta polypeptide 1 | 2782 |
| GNB3 | guanine nucleotide binding protein (G protein), beta polypeptide 3 | 2784 |
| GNG11 | guanine nucleotide binding protein (G protein), gamma 11 | 2791 |
| GNG2 | guanine nucleotide binding protein (G protein), gamma 2 | 54331 |
| GNG3 | guanine nucleotide binding protein (G protein), gamma 3 | 2785 |
| GNG4 | guanine nucleotide binding protein (G protein), gamma 4 | 2786 |
| GNGT1 | guanine nucleotide binding protein (G protein), gamma transducing activity polypeptide 1 | 2792 |
| GNGT2 | guanine nucleotide binding protein (G protein), gamma transducing activity polypeptide 2 | 2793 |
| GOLGA5 | golgi autoantigen, golgin subfamily a, | 9950 |
| GPRK6 | G protein-coupled receptor kinase 6 | 2870 |
| GRB10 | growth factor receptor-bound protein 10 | 2887 |
| GRB2 | growth factor receptor-bound protein 2 | 2885 |
| GSK3B | glycogen synthase kinase 3 beta | 2932 |
| HBG1 | hemoglobin, gamma A | 3047 |

| Symbol | Gene Name | Entrez Gene ID |
|---|---|---|
| HCLS1 | hematopoietic cell-specific Lyn substrate 1 | 3059 |
| HIF1A | hypoxia-inducible factor 1, alpha subunit | 3091 |
| HIF3A | hypoxia inducible factor 3, alpha subunit | 64344 |
| HIST1H4I | histone 1, H4i | 8294 |
| HK1 | hexokinase 1 | 3098 |
| HMGB2 | high-mobility group box 2 | 3148 |
| HPRT1 | hypoxanthine phosphoribosyltransferase 1 (Lesch-Nyhan syndrome) | 3251 |
| HRAS | v-Ha-ras Harvey rat sarcoma viral oncogene homolog | 3265 |
| HSPA1A | heat shock 70kDa protein 1A | 3303 |
| HSPCA | heat shock 90kDa protein 1, alpha | 3320 |
| HUS1 | HUS1 checkpoint homolog | 3364 |
| ID1 | inhibitor of DNA binding 1, dominant negative helix-loop-helix protein | 3397 |
| ID2 | inhibitor of DNA binding 2, dominant negative helix-loop-helix protein | 3398 |
| IKBKB | inhibitor of kappa light polypeptide gene enhancer in B-cells | 3551 |
| IL18 | interleukin 18 | 3606 |
| ILK | integrin-linked kinase | 3611 |
| IMPDH1 | IMP (inosine monophosphate) dehydrogenase 1 | 3614 |
| INDO | indoleamine-pyrrole 2,3 dioxygenase | 3620 |
| INHBA | inhibin, beta A (activin A, activin AB alpha polypeptide) | 3624 |
| IRF5 | interferon regulatory factor 5 | 3663 |
| ISG20 | interferon stimulated gene 20kDa | 3669 |
| JIK | STE20-like kinase | 51347 |
| KRAS2 | v-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog | 3845 |
| LCK | lymphocyte-specific protein tyrosine kinase | 3932 |
| LIMK2 | LIM domain kinase 2 | 3985 |
| MADH2 | MAD, mothers against decapentaplegic homolog 2 | 4087 |
| MADH4 | MAD, mothers against decapentaplegic homolog 4 | 4089 |
| MADH5 | MAD, mothers against decapentaplegic homolog 5 | 4090 |
| MADH9 | MAD, mothers against decapentaplegic homolog 9 | 4093 |
| MAP2K3 | mitogen-activated protein kinase kinase 3 | 5606 |
| MAP2K5 | mitogen-activated protein kinase kinase 5 | 5607 |
| MAP2K6 | mitogen-activated protein kinase kinase 6 | 5608 |
| MAP3K6 | mitogen-activated protein kinase kinase kinase 6 | 9064 |
| MAP3K7 | mitogen-activated protein kinase kinase kinase 7 | 6885 |
| MAPK1 | mitogen-activated protein kinase 1 | 5594 |
| MAPK11 | mitogen-activated protein kinase 11 | 5600 |
| MAPK13 | mitogen-activated protein kinase 13 | 5603 |

| Symbol | Gene Name | Entrez Gene ID |
|---|---|---|
| MAPK14 | mitogen-activated protein kinase 14 | 1432 |
| 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 |
| MATK | megakaryocyte-associated tyrosine kinase | 4145 |
| MAX | MAX protein | 4149 |
| MCM5 | MCM5 minichromosome maintenance deficient 5 | 4174 |
| MEF2A | MADS box transcription enhancer factor 2, polypeptide A | 4205 |
| MEF2C | MADS box transcription enhancer factor 2, polypeptide C | 4208 |
| MIF | macrophage migration inhibitory factor | 4282 |
| MKNK1 | MAP kinase-interacting serine/threonine kinase 1 | 8569 |
| MMP2 | matrix metalloproteinase 2 | 4313 |
| MTCP1 | mature T-cell proliferation 1 | 4515 |
| MYBL2 | v-myb myeloblastosis viral oncogene homolog (avian)-like 2 | 4605 |
| MYD88 | myeloid differentiation primary response gene (88) | 4615 |
| NCK1 | NCK adaptor protein 1 | 4690 |
| NCOA4 | nuclear receptor coactivator 4 | 8031 |
| NFKBIA | nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor | 4792 |
| NR1I2 | nuclear receptor subfamily 1, group I, member 2 | 8856 |
| NR3C1 | nuclear receptor subfamily 3, group C, member 1 | 2908 |
| NR4A1 | nuclear receptor subfamily 4, group A, member 1 | 3164 |
| NR4A2 | nuclear receptor subfamily 4, group A, member 2 | 4929 |
| NUDT2 | nudix (nucleoside diphosphate linked moiety X)-type motif 2 | 318 |
| PAK4 | p21(CDKN1A)-activated kinase 4 | 10298 |
| PDE4A | phosphodiesterase 4A, cAMP-specific | 5141 |
| PDK1 | pyruvate dehydrogenase kinase, isoenzyme 1 | 5163 |
| PIK3R1 | phosphoinositide-3-kinase, regulatory subunit, polypeptide 1 (p85 alpha) | 5295 |
| PLD2 | phospholipase D2 | 5338 |
| PPARG | peroxisome proliferative activated receptor | 5468 |
| PPM1A | protein phosphatase 1A (formerly 2C), magnesium-dependent, alpha | 5494 |
| PPP2R2B | protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), beta | 5521 |
| PPP2R2C | protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), gamma | 5522 |
| PPP2R5A | protein phosphatase 2, regulatory subunit B (B56), alpha | 5525 |
| PPP2R5C | protein phosphatase 2, regulatory subunit B (B56), gamma | 5527 |
| PRDM4 | PR domain containing 4 | 11108 |
| PRKACB | protein kinase, cAMP-dependent, catalytic, beta | 5567 |

| Symbol | Gene Name | Entrez Gene ID |
|---|---|---|
| PRKAR1A | protein kinase, cAMP-dependent, regulatory, type I, alpha | 5573 |
| 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 |
| PTPN1 | protein tyrosine phosphatase, non-receptor type 1 | 5770 |
| PTPN11 | protein tyrosine phosphatase, non-receptor type 11 | 5781 |
| PXK | PX domain containing serine/threonine kinase | 54899 |
| RAC1 | ras-related C3 botulinum toxin substrate 1 | 5879 |
| RAC2 | ras-related C3 botulinum toxin substrate 2 | 5880 |
| RAF1 | v-raf-1 murine leukemia viral oncogene homolog 1 | 5894 |
| RARA | retinoic acid receptor | 5914 |
| RET | ret proto-oncogene | 5979 |
| RHOH | ras homolog gene family, member H | 399 |
| 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 |
| RRAS | related RAS viral (r-ras) oncogene homolog | 6237 |
| RRAS2 | related RAS viral (r-ras) oncogene homolog 2 | 22800 |
| SAV1 | salvador homolog 1 | 60485 |
| SGKL | serum/glucocorticoid regulated kinase-like | 23678 |
| SH2B | SH2-B homolog | 25970 |
| SLA | src-like-adaptor | 6503 |
| SNK | serum-inducible kinase | 10769 |
| SOCS2 | suppressor of cytokine signaling 2 | 8835 |
| SOCS4 | suppressor of cytokine signaling 4 | 9306 |
| SOCS5 | suppressor of cytokine signaling 5 | 9655 |
| SPARC | secreted protein, acidic, cysteine-rich (osteonectin) | 6678 |
| SRC | v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian) | 6714 |
| STAM | signal transducing adaptor molecule (SH3 domain and ITAM motif) 1 | 8027 |
| STAT1 | signal transducer and activator of transcription 1, 91kDa | 6772 |
| STAT3 | signal transducer and activator of transcription 3 | 6774 |
| STAT4 | signal transducer and activator of transcription 4 | 6775 |

| Symbol | Gene Name | Entrez Gene ID |
|---|---|---|
| STAT5A | signal transducer and activator of transcription 5A | 6776 |
| STK17B | serine/threonine kinase 17b (apoptosis-inducing) | 9262 |
| STK22C | serine/threonine kinase 22C (spermiogenesis associated) | 81629 |
| STK24 | serine/threonine kinase 24 | 8428 |
| STK25 | serine/threonine kinase 25 | 10494 |
| STK3 | serine/threonine kinase 3 | 6788 |
| STK38 | serine/threonine kinase 38 | 11329 |
| STK38L | serine/threonine kinase 38 like | 23012 |
| STK6 | serine/threonine kinase 6 | 6790 |
| STMN1 | stathmin 1/oncoprotein 18 | 3925 |
| SYK | spleen tyrosine kinase | 6850 |
| TBK1 | TANK-binding kinase 1 | 29110 |
| TBL1X | transducin (beta)-like 1X-linked | 6907 |
| TEK | TEK tyrosine kinase, endothelial | 7010 |
| TFG | TRK-fused gene | 10342 |
| TLK1 | tousled-like kinase 1 | 9874 |
| TNFRSF6 | tumor necrosis factor receptor superfamily | 355 |
| TOLLIP | toll interacting protein | 54472 |
| TRAF2 | TNF receptor-associated factor 2 | 7186 |
| TRAF4 | TNF receptor-associated factor 4 | 9618 |
| TRAF5 | TNF receptor-associated factor 5 | 7188 |
| TRAF6 | TNF receptor-associated factor 6 | 7189 |
| TRAP100 | thyroid hormone receptor-associated protein | 9862 |
| TRAP25 | TRAP/Mediator complex component | 90390 |
| TTRAP | TRAF and TNF receptor associated protein | 51567 |
| VAV1 | vav 1 oncogene | 7409 |
| VDRIP | vitamin D receptor interacting protein | 29079 |
| VEGFB | vascular endothelial growth factor B | 7423 |
| ZAK | sterile alpha motif and leucine zipper containing kinase AZK | 51776 |
| ZAP70 | zeta-chain (TCR) associated protein kinase | 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-$CONH_2$) 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-KVEKIGEGTY*GVVKK-$CONH_2$) that is recognized by anti-phosphotyrosine antibodies.

| 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 |

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# Relevant Patents


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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

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