

**Panorama™
Human Protein
Function Microarray Cancer v1**

Technical Bulletin

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Panorama Human Protein Function Microarray Cancer v1

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Introduction

The Panorama Human Protein Function Microarray Cancer v1 contains a set of 130 known human cancer proteins and specific controls. The protein content is based on "A Census of Human Cancer Genes," published in 2004 by Michael R. Stratton and co-workers from the Wellcome Trust Sanger Institute in Hinxton, UK¹. The proteins encoded by these genes regulate processes such as cell proliferation, cell differentiation, cell death, and DNA repair. Spotted cancer proteins were expressed in Sf9 insect cells and affinity purified directly on the array via their biotin tag. The functional proteins are oriented in the same manner on the microarray allowing evaluation of their function in a parallel format.

Panorama Array Technology

Panorama functional protein arrays utilize a proprietary BCCP (biotin-carboxyl carrier protein) tagging technology to ensure only correctly folded and fully functional proteins are arrayed^{2,3,4}. Open reading frames (ORFs) are cloned in frame with sequences encoding the BCCP and the c-Myc epitope (EQKLISEEDL of the c-Myc oncoprotein; SwissProt accession number: MYC_HUMAN), which can be used to visualize the proteins on the array. To ensure fidelity, clones are sequence-verified immediately prior to expression in insect cells.

During expression, the BCCP tag is biotinylated only when it is correctly folded. There is inter-dependence between tag folding and protein folding. Biotinylation is a marker of correct protein folding^{2,5} and ensures only desired proteins are affinity purified. Additionally, all expressed constructs are assayed for incorporation of biotin. Western blot analysis is performed with each protein 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. 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, which have been reported for other types of array surfaces⁶.

Panorama functional protein arrays are fabricated on borosilicate glass slides (25 × 75.6 × 1 mm) 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 and suitable for screening with a range of molecules including nucleic acids, proteins, and small molecules.

Kit Contents

The Cancer Array v1 kit consists of:

Product	Cat No.	Size
Panorama Human Cancer v1 Microarrays	C9116	2 each
Calmodulin-Cy5	C9741	15 μ L
Anti-c-Myc-Cy3	C6594	10 μ L
Assay Buffer	A1105	150 mL
Calmodulin Buffer	C0492	150 mL
Bovine Serum Albumin (BSA)	A3059	200 mg
1 M Dithiothreitol (DTT)	646563	1 ampule
quadriPERM [®] Culture Vessel	Z376760	2 each
HybriSlip [™]	H0784	10 each
Pap Jar	P8123	4 each
50 mL Conical Centrifuge Tubes	C8296	2 each
Panorama Cancer Array v1 analysis workbook and gal file (diskette)	C0367	1 each

Not included with the human cancer protein array:

The following materials are not included in the protein array kit, but are required to perform the calmodulin 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
- Orbital shaker

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.

On receipt, the kit contents must be placed at $-20\text{ }^{\circ}\text{C}$ until use. The storage buffer for the protein arrays may be frozen on arrival due to the dry ice used for shipping, but will thaw gently when placed at $-20\text{ }^{\circ}\text{C}$. 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 Prior to Using the Kit

- 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 upward when lying flat. Wash the array as indicated in the protocols to remove components of the storage buffer that may affect assay performance.
- d. Cover arrays completely in assay buffer/reagents to prevent them from drying out during the assay.
- e. If using critical volume sample, use HybriSlips 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 aluminium foil.
- g. Where compatible with assay conditions and detection methods, include 20% glycerol and 0.1% Triton X-100 in buffers.
- h. The Cancer Array v1 has been pre-blocked with 2% BSA during production. Additional blocking with non-fat milk powder or other commonly used blocking agents may be necessary for specific applications (see below).
- i. If a high degree of background speckling is observed after processing, use de-speckling algorithms, which 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.

Description of Major Kit Components

Protein Arrays

Each Cancer Array v1 contains 130 wild-type cancer proteins, 10 control features, and markers in 48 sub-grids per slide. The cancer proteins within the arrays have been printed in quadruplicate. Each feature on the array has a spot diameter of approximately 500 μm spaced at intervals of 300 μm . Optimized spotting protocols result in uniform spot morphology and even protein deposition across each spot. The arrayed cancer 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 of the Cancer Array v1 are shown in Appendices A and B. All proteins are expressed in insect cells and have been analyzed by Western blot.

Each Cancer Array v1 contains:

- 130 human cancer 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 fused with the immobilization tag, BCCP
- 4 anti-c-Myc positive control spots consisting of β -galactosidase fused with BCCP-c-Myc
- 4 anti-c-Myc positive control spots consisting of β -galactosidase fused with the immobilization tag, BCCP-Myc
- A dilution series of 12 anti-c-Myc positive control spots consisting of β -galactosidase fused with the immobilization tag, BCCP-Myc
- A dilution series of 12 negative control spots consisting of biotin-BSA
- Negative control spots printed with Assay Buffer
- 4 positive control spots containing calcium calmodulin dependent kinase IV (CAMK4)

The proteins are arrayed onto a streptavidin-coated, low fluorescence glass slide ($25 \times 75.6 \times 1 \text{ mm}$) that is compatible with conventional microarray instrumentation. The upper side and orientation is indicated by the label (Appendix A). The arrays are pre-washed and blocked with 2% BSA to reduce non-specific binding. Additional specific blocking may be required, depending on the probing solutions to be used (see Assay Guidelines, pg 13).

Calmodulin-Cy5

A protein:protein binding assay using Calmodulin-Cy5 is recommended as a control assay to test the functionality of proteins on the Cancer Array v1. For this control assay, a Cy5-labeled calmodulin probe is provided in the kit. In the presence of free calcium, calmodulin will bind specifically to the calcium/calmodulin dependent protein kinase (CAMK4) that is present on the array as a positive control. The specificity of calmodulin binding to CAMK4 can be investigated by measuring binding in the presence of excess EGTA. In the absence

of free calcium, binding of calmodulin to CAMK4 is inhibited. The binding assay must be carried out using the Calmodulin Buffer provided with the kit. A typical image showing calmodulin binding to the different cancer proteins on the array is shown in Figure 1, pg 10.

Anti-c-Myc-Cy3

An anti-c-Myc-Cy3 monoclonal antibody is provided and serves as a positive control because the c-Myc tag is incorporated into all cancer proteins on the array. It is recommended to use the anti-c-Myc-Cy3 to probe each control or experimental array after performing primary assays for protein function. This assay demonstrates that proteins are immobilized at each spot, which can be used to quantify the amount of protein and normalize data (see below) after assay. The binding assay must be carried out using the Assay Buffer that is provided with the kit. A typical image showing anti-c-Myc-Cy3 antibody binding is shown in Figure 2, pg 11.

Recommended Control Assays

Protocol for Calmodulin-Cy5 Binding Assay

- a. Prior to performing the Calmodulin-Cy5 binding assay, add 150 μ L of 1 M DTT (provided with the kit) to the Calmodulin Buffer and place on ice.
- b. Remove array from storage buffer and place in a clean Pap jar containing 25 mL of Calmodulin Buffer. Place the Pap jar on ice and shake gently for 5 minutes.
- c. Dilute the Calmodulin-Cy5 1:1000 in ice-cold Calmodulin Buffer (12 μ L of stock reagent added to 12 mL of Calmodulin Buffer; final concentration 30 nM).
- d. Remove the array from the wash solution and drain the slide by blotting the long edge carefully onto lint-free tissue paper for 10 seconds.
- e. Dry the back of the slide with lint-free tissue paper.
- f. Place the slide into a chamber of a quadriPERM culture dish. When using quadriPERM dishes ensure the slide does not rest on the plastic lugs at the numbered end of the chamber. This may lead to incomplete coverage of the slide by the probing solution. Immediately pipette 5 mL of the diluted Calmodulin-Cy5 solution carefully onto the slide, while minimizing introduction of bubbles. Add additional slides to the chambers in the same manner, then replace the lid.
- g. Place the chamber on an orbital shaker in a 4 °C cold room, refrigerator, or on ice, but ensure the quadriPERM dish is horizontal so the probing solution covers the entire array. To protect the fluor, cover the quadriPERM vessel with foil. Shake gently for 30 minutes.
- h. Perform all incubations using Cy-dyes away from direct light. The chamber may be covered with foil during incubations.
- i. 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 ice-cold Calmodulin Buffer. Cap the tube, then **invert** several times. Place the tube in ice and shake gently for 5 minutes to remove unbound probe. Pour off the Calmodulin Buffer.

- j. Repeat the wash two more times, **inverting** the container several times at each wash step.
- k. Pour off the final Calmodulin Buffer and add 25 mL of ice-cold high purity water to wash away glycerol. Replace the lid on the container and **invert** several times before pouring off the water.
- l. 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 4 °C for 2 minutes at $240 \times g$.
- m. Carefully remove the slides from the centrifuge tubes with a pair of dry, blunt-ended forceps, touching only the extreme end of the slide.
- n. 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 described above, use the anti-c-Myc-Cy3 antibody to demonstrate protein is present in each cancer 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**: add 150 μ L of 1 M DTT to 150 mL of Assay Buffer immediately before use.
- c. Prepare 10 mL of **anti-c-Myc Probing Buffer**: add 200 mg of BSA to 10 mL of anti-c-Myc Wash Buffer from step c.
- d. Dilute the anti-c-Myc-Cy3 conjugate 1:1000 (5 μ L anti-c-Myc-Cy3 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 and incubate with gentle shaking 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 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, then replace the lid. When using quadriPERM dishes, ensure the slide does not rest on the plastic lugs at the numbered end of the chamber. This may lead to incomplete coverage of the slide by the probing solution.
- i. Check that the quadriPERM dish is horizontal so the probing solution covers the entire array. To protect the floor, cover the quadriPERM vessel with foil.

- j. Incubate 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, but to increase the assay sensitivity the array can be incubated overnight up to 18 hours before processing. For longer incubations, place the quadriPERM dish in a sealed plastic box together with a wad of damp tissues to prevent drying during incubation.
- k. 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 (two arrays per tube). Cap the Pap jar, then **invert** several times. Shake gently for 5 minutes to remove unbound antibody. Pour off the anti-c-Myc Wash Buffer.
- l. Repeat two more times, **inverting** the Pap jar several times at each wash step.
- m. Pour off the final anti-c-Myc Wash Buffer and add 25 mL of water to the Pap jar. Cap the tube and **invert** several times to remove glycerol from the slide surface before drying the slide by centrifugation.
- n. 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 4 °C for 2 minutes at $240 \times g$.
- o. Carefully remove the slides from the centrifuge tubes with a pair of blunt-ended forceps touching only the extreme end of the slide.
- p. 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. Appropriate 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 lower PMT settings. Scanning should be repeated to obtain an optimal image bearing in mind that "overexposure" can lead to photobleaching of fluorors.

- e. Images should be saved as tif files for future analysis.

Data Analysis

Analyze the images using a standard microarray analysis software package. After loading the tif file, the array can be analyzed 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. Cut and paste the column of data for each slide into the blue columns in the Cy3 or Cy5 "Input" sheets of the Panorama Cancer Array v1 analysis Excel spreadsheet provided with the kit. 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.

Interpretation of the Calmodulin-Cy5 Binding Assay

Shown in Figure 1 is a typical image of a Panorama Cancer Array v1 after performing the Calmodulin-Cy5 binding assay. A detail of the image shows binding of Calmodulin-Cy5 to the panel of proteins including CAMK4. CAMK4 is a calmodulin dependent protein kinase. Binding of calmodulin by CAMK4 is calcium-dependent, and this can be demonstrated on the arrays by performing assays in the presence or absence of calcium. Minor binding of Calmodulin-Cy5 to some other proteins on the array is not calcium dependent.

Determine the identity and coordinates of each protein feature by referring to the gal file present on the Sigma protein array Web site (sigma-aldrich.com) and supplied on the diskette with this kit.

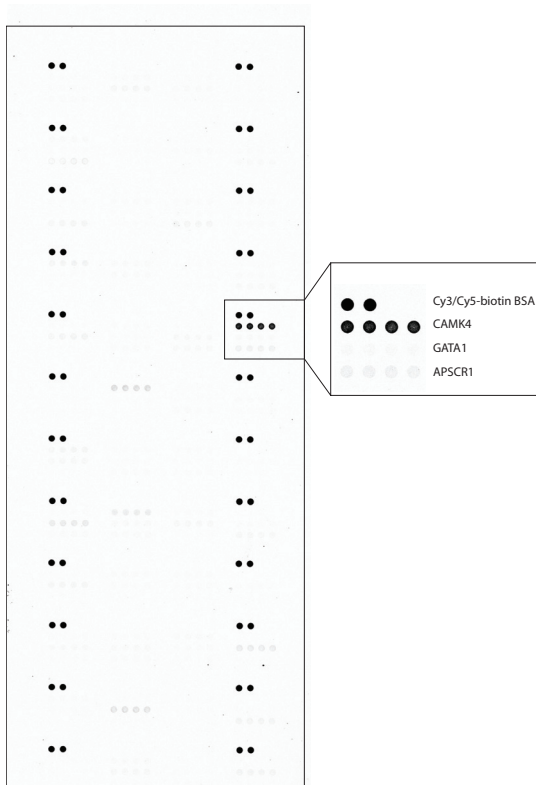


Figure 1. Panorama Human Cancer Array v1: Image showing Calmodulin-Cy5 binding to entire array. Paired spots in the left and right columns of sub-panels are Cy3/Cy5-labeled biotin BSA marker proteins. Boxed area shows the panel containing CAMK4. Detail of boxed area from entire array demonstrates binding of Calmodulin-Cy5 to the panel containing four replicates of CAMK4. Array not drawn to scale.

Interpretation of the Anti-c-Myc-Cy3 Binding Assay

Analyze the images obtained after carrying out the anti-c-Myc-Cy3 binding assay in the same way as analysis of Calmodulin-Cy5 binding.

Figure 2 shows a typical image of a Panorama Cancer Array v1 after assay with anti-c-Myc-Cy3. Features which do not exhibit anti-c-Myc-Cy3 binding are controls and a small number of proteins which are not visualized in this assay, but have been detected in Western blots

of insect cell lysates. The number of proteins visualized in this assay will vary depending on the assay that is performed. The identity and coordinates of each protein feature can be determined by referring to the gal file present on the Sigma protein array Web site (sigma-aldrich.com) and supplied on the diskette with this kit.

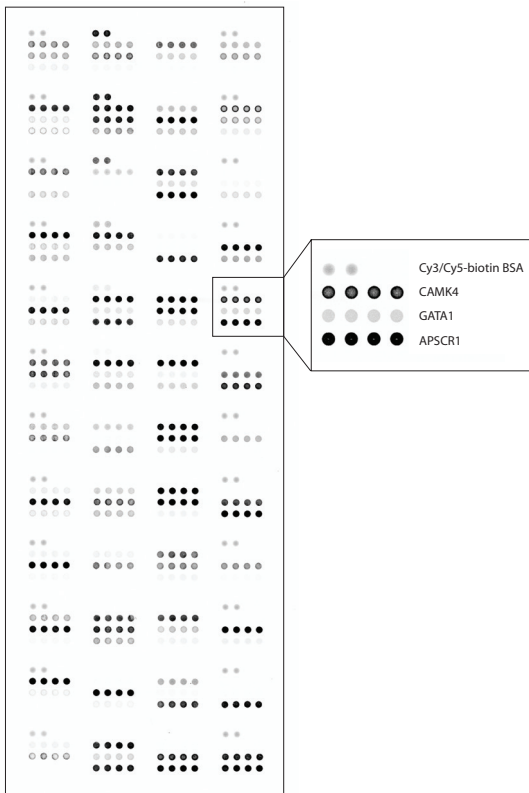


Figure 2. Panorama Human Cancer Array v1: Image showing anti-c-Myc-Cy3 binding to entire array after 18 hours incubation at room temperature. Paired spots in the left and right columns of sub-panels are Cy3/Cy5-labeled biotin BSA marker proteins. Boxed area shows the features corresponding to the box marked. Detail of boxed area from entire array demonstrates binding of anti-c-Myc-Cy3 to the panel containing CAMK4. Array not drawn to scale.

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 Cancer Array v1 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.

These 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 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, electrophoretic mobility shift, or solution assays.

Guidelines for Different Assay Types

General Recommendations

The buffers provided for the control assays supplied with this kit may not be suitable for all assays.

Design buffers in accordance with the known literature for the particular assay under investigation taking due consideration of the need for particular ions or cofactors. 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 provided they are compatible with the assay and detection conditions.

Cancer Array v1 has been blocked with 2% BSA; however, depending on the assay, it may be necessary to apply further blocking. 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. 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 that there is minimal interference with binding.

Label peptide and oligonucleotide probes during synthesis and purify them by 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 $-20\text{ }^{\circ}\text{C}$. Do not freeze/thaw aliquots of probes more than once.

Where applicable, it is recommended to perform assays at $4\text{ }^{\circ}\text{C}$ to maximize protein stability.

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 $-20\text{ }^{\circ}\text{C}$. 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 $-20\text{ }^{\circ}\text{C}$. 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.

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 (i.e., protein interactions).

The proteins on the array can be modified enzymatically on the array and detected using labeled antibodies specific for a particular modification (i.e., tyrosine phosphorylation can be detected using anti-phosphotyrosine antibodies).

Troubleshooting Guide

Calmodulin binding to CAMK4 is not detected during the Calmodulin-Cy5 binding assay

- Re-scan the array at a higher laser power or PMT voltage.
- Be certain incubation times were followed correctly and wash steps were carried out in accordance with the protocol.

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 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 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.
- Verify the presence of cancer proteins in each spot using the anti-c-Myc-Cy3 antibody.

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 take care 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:** This 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 dependent. 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 and not allowed to dry out during assay or processing.

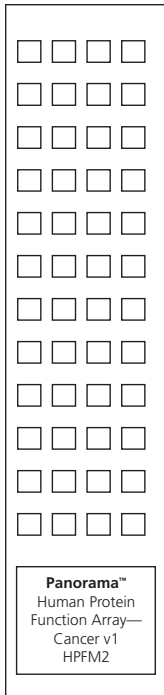
Quality Control

The proteins included on the array are produced from fully sequence-verified clones and expressed in insect cells infected with recombinant baculovirus. Prior to array manufacturing, recombinant protein expression is verified by Western blotting analysis.

Quality control tests are performed to check the performance of the cancer protein function arrays. For example, each slide is inspected visually for imperfections that are known to affect experimental results. Each batch undergoes tests that measure the performance of the proteins on the array, including the functional Calmodulin-Cy5 binding assay and the anti-c-Myc-Cy3 binding assay.

Appendices

Appendix A: Protein Array Orientation



The Human Cancer Array v1 consists of 48, 4×4 sub-arrays. 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 sub-panels. A dilution series of control proteins are situated in the top line of the second column of sub-arrays. For information regarding protein layout, a gal file is available on the Sigma-Aldrich Web site (sigma-aldrich.com). Array not drawn to scale.

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

Symbol	Gene Name	Entrez Gene ID
ABI1	spectrin SH3 domain binding protein 1	10006
ABL2	v-abl Abelson murine leukemia viral oncogene homolog 2 (arg Abelson-related gene)	27
ACSL6	acyl-CoA synthetase long-chain family member 6	23305
AF1Q	ALL1-fused gene from chromosome 1q	10962
AF5Q31	ALL1 fused gene from 5q31	27125
AKT1	v-akt murine thymoma viral oncogene homolog 1	207
ARNT	aryl hydrocarbon receptor nuclear translocator	405
ASPSCR1	alveolar soft part sarcoma chromosome region candidate 1	79058
ATF1	activating transcription factor 1	466
ATIC	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase	471
BCL10	B-cell CLL/lymphoma 10	8915
BFHD	Beukes familial hip dysplasia	50947
BIRC3	baculoviral IAP repeat-containing 3	330
BMPR1A	bone morphogenetic protein receptor type IA	657
BTG1	B-cell translocation gene 1 anti-proliferative	694
CBFA2T1	core-binding factor runt domain alpha subunit 2 translocated to 1 cyclin D-related	862
CBFA2T3	core-binding factor runt domain alpha subunit 2 translocated to 3	863
CBFB	core-binding factor beta subunit	865
CCND1	cyclin D1 (PRAD1: parathyroid adenomatosis 1)	595
CDC2	cell division cycle 2 G1 to S and G2 to M	983
CDK4	cyclin-dependent kinase 4	1019
CHIC2	cysteine-rich hydrophobic domain 2	26511
CHN1	chimerin (chimaerin) 1	1123
COPEB	core promoter element binding protein	1316

Symbol	Gene Name	Entrez Gene ID
COX6C	cytochrome c oxidase subunit VIc	1345
CTNNB1	catenin (cadherin-associated protein) beta 1 88kDa	1499
CYLD	cyldromatosis (turban tumor syndrome)	1540
DDB2	damage-specific DNA binding protein 2 48kDa	1643
DDIT3	DNA-damage-inducible transcript 3	1649
DEK	DEK oncogene (DNA binding)	7913
EIF4A2	eukaryotic translation initiation factor 4A isoform 2	1974
EPS15	epidermal growth factor receptor pathway substrate 15	2060
ERCC2	excision repair cross-complementing rodent repair deficiency complementation group 2 (xeroderma pigmentosum D)	2068
ERCC3	excision repair cross-complementing rodent repair deficiency complementation group 3 (xeroderma pigmentosum group B complementing)	2071
ERCC5	excision repair cross-complementing rodent repair deficiency complementation group 5 (xeroderma pigmentosum complementation group G (Cockayne syndrome))	2073
ERG	v-ets erythroblastosis virus E26 oncogene like (avian)	2078
ETV4	ets variant gene 4 (E1A enhancer binding protein E1AF)	2118
ETV6	ets variant gene 6 (TEL oncogene)	2120
EWSR1	Ewing sarcoma breakpoint region 1	2130
EXT1	exostoses (multiple) 1	2131
EXT2	exostoses (multiple) 2	2132
FANCC	Fanconi anemia complementation group C	2176
FANCG	Fanconi anemia complementation group G	2189
FGFR10P	FGFR1 oncogene partner	11116
FH	fumarate hydratase	2271
FIP1L1	FIP1 like 1 (<i>S. cerevisiae</i>)	81608
FUS	fusion (involved in t(12 16) in malignant liposarcoma)	2521
GAS7	growth arrest-specific 7	8522

Symbol	Gene Name	Entrez Gene ID
GATA1	GATA binding protein 1 (globin transcription factor 1)	2623
GMPS	guanine monphosphate synthetase	8833
GOLGA5	golgi autoantigen golgin subfamily a 5	9950
GPC	glypican 3	2719
GPHN	gephyrin	10243
HIST1H4I	histone 1 H4i	8294
HRAS	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	3265
HSPCA	heat shock 90kDa protein 1 alpha	3320
IL21R	interleukin 21 receptor	50615
IRF4	interferon regulatory factor 4	3662
KRAS2	v-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog	3845
LASP1	LIM and SH3 protein 1	3927
LCP1	lymphocyte cytosolic protein 1 (L-plastin)	3936
LHFP	lipoma HMGIC fusion partner	10186
LMO2	LIM domain only 2 (rhombotin-like 1)	4005
LYL1	lymphoblastic leukemia derived sequence 1	4066
MADH4	MAD (mothers against decapentaplegic) homolog 4 (<i>Drosophila</i>)	4089
MLF1	myeloid leukemia factor 1	4291
MLH1	mutL homolog 1 colon cancer nonpolyposis type 2 (<i>E. coli</i>)	4292
MLLT3	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog <i>Drosophila</i>) translocated to 3	4300
MLLT6	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog <i>Drosophila</i>) translocated to 6	4302
MNAT1	menage a trois 1 (CAK assembly factor)	4331
MSF	MLL septin-like fusion	10801
MSH2	mutS homolog 2 colon cancer nonpolyposis type 1 (<i>E. coli</i>)	4436
MSN	moesin	4478
MUTYH	mutY homolog (<i>E. coli</i>)	4595

Symbol	Gene Name	Entrez Gene ID
MYC	v-myc myelocytomatosis viral oncogene homolog (avian)	4609
NCOA4	nuclear receptor coactivator 4	8031
NF2	neurofibromin 2 (bilateral acoustic neuroma)	4771
NPM1	nucleophosmin (nucleolar phosphoprotein B23 numatrin)	4869
NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog	4893
PAX8	paired box gene 8	7849
PCBD	6-pyruvoyl-tetrahydropterin synthase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1)	5092
PDGFB	platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) oncogene homolog)	5155
PIM1	pim-1 oncogene	5292
PLK2	polo-like kinase 2	10769
PNUTL1	peanut-like 1	5413
POU2F1	POU domain class 2 associating factor 1	5450
PPARG	peroxisome proliferative activated receptor gamma	5468
PRCC	papillary renal cell carcinoma (translocation-associated)	5546
PRKACB	protein kinase cAMP-dependent catalytic beta	5567
PRKAR1A	protein kinase cAMP-dependent regulatory type I alpha (tissue specific extinguisher 1)	5573
PTEN	phosphatase and tensin homolog (mutated in multiple advanced cancers 1)	5728
PTPN11	protein tyrosine phosphatase non-receptor type 11 (Noonan syndrome 1)	5781
RABEP1	rabaptin RAB GTPase binding effector protein 1	9135
RAD51L1	RAD51-like 1 (<i>S. cerevisiae</i>)	5890
RAP1GDS1	RAP1 GTP-GDP dissociation stimulator 1	5910
RARA	retinoic acid receptor alpha	5914
RB1	retinoblastoma 1	5925

Symbol	Gene Name	Entrez Gene ID
RET	ret proto-oncogene (multiple endocrine neoplasia and medullary thyroid carcinoma 1 Hirschsprung disease)	5979
RHOH	ras homolog gene family member H	399
RPL22	ribosomal protein L22	6146
SBDS	Shwachman-Bodian-Diamond syndrome	51119
SDHB	succinate dehydrogenase complex subunit B iron sulfur (lp)	6390
SEPTIN6	septin 6	23157
SET	SET translocation (myeloid leukemia-associated)	6418
SH3GL1	SH3-domain GRB2-like 1	6455
SS18L1	synovial sarcoma translocation gene on chromosome 18-like 1	26039
SSX1	synovial sarcoma X breakpoint 1	6756
SSX2	synovial sarcoma X breakpoint 2	6757
SSX4	synovial sarcoma X breakpoint 4	6759
STAT3	signal transducer and activator of transcription 3 (acute-phase response factor)	6774
TAF15	TAF15 RNA polymerase II TATA box binding protein (TBP)-associated factor 68kDa	8148
TCF12	transcription factor 12 (HTF4 helix-loop-helix transcription factors 4)	6938
TCL1A	T-cell leukemia/lymphoma 1A	8115
TFE3	transcription factor binding to IGHM enhancer 3	7030
TFEB	transcription factor EB	7942
TFG	TRK-fused gene	10342
TFPT	TCF3 (E2A) fusion partner (in childhood Leukemia)	29844
TFRC	transferrin receptor (p90 CD71)	7037
TNFRSF6	tumor necrosis factor receptor superfamily member 6	355
TP53	p53 tumor suppressor	7157
TPM3	tropomyosin 3	7170
TPM4	tropomyosin 4	7171

Symbol	Gene Name	Entrez Gene ID
TRIP11	thyroid hormone receptor interactor 11	9321
VHL	von Hippel-Lindau syndrome	7428
WAS	Wiskott-Aldrich syndrome (eczema-thrombocytopenia)	7454
WT1	Wilms tumor 1	7490
ZNF198	zinc finger protein 198	7750
ZNF278	zinc finger protein 278	23598
ZNF384	zinc finger protein 384	171017
ZNFN1A1	zinc finger protein subfamily 1A 1 (Ikaros)	10320

Control and Marker Proteins

Protein Content Code	Identify
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
Control 5	calcium/calmodulin-dependent protein kinase IV (CAMK4)
Control 6	Assay buffer
Control 7	Assay buffer
Control 8	Assay buffer
Control 9	Assay buffer
Control 10	Assay buffer
Control 11	Assay buffer
Control 12	Assay 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.

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

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

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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

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