

ProteoScan Cancer Lysate Array

A high-density quantitative Reverse Phase Protein Array for biomarkers research

Application Guide

Table of Contents

Package Contents and Storage Conditions	2
Package Contents.....	2
Storage Conditions.....	2
Other Required Materials and Reagents.....	2
Reagents (Recommended reagents).....	2
Equipment and Instruments	3
Introduction	4
Array Layouts	5
Array controls	7
Technical Notes	8
Experimental Procedures:.....	9
General Protocol	9
Detection Methods	10
Tyramide Signal Amplification (TSA) enhancement protocols	10
Data Analysis	11
Appendix A.....	12
Quality of biospecimens	12
Protein Extraction.....	12
Array Printing	12
Frequently Asked Questions.....	13

Package Contents and Storage Conditions

Package Contents

SKU PA100002 ProteoScan Cancer Lysate Array 1.0

- 2 ProteoScan Cancer Lysate Array slides with a total of 6,603 features (spots) each.
 - The array slide is a 1 x 3" (25mm x 75mm) glass slide coated with a thin layer of nitrocellulose (length: 60 mm). A small sticker located on the nitrocellulose side provides a unique barcode number for identification of each array. The array is shipped in a round plastic protective container.
- ProteoScan Cancer Lysate Array Application Guide

SKU PA100001 ProteoScan Assay Validation Array 1.0

- 2 ProteoScan Assay Validation Array slides with a total of 201 features (spots) each.
 - The array slide is a 1 x 3" (25mm x 75mm) glass slide coated with a thin layer of nitrocellulose (length: 51 mm). A small sticker with the product name is located on the nitrocellulose side. The array is shipped in a round plastic protective container.
- ProteoScan Cancer Lysate Array Application Guide

Storage Conditions

The Arrays are shipped in a round plastic protective container at room temperature and should be moved to cold storage (-20°C) immediately upon arrival. The array must be kept dry but the addition of desiccant is not recommended. Wrap the head of the protective container with parafilm and place the array in a dry box at -20°C for long-term storage.

This product is for research use only. Use in and/or for diagnostic or therapeutic purposes is strictly prohibited.

Other Required Materials and Reagents

Reagents (Recommended reagents)

- Washing buffer TBST. (20 mM Tris, 150 mM NaCl, 0.05-0.1% Tween20, pH 7.5).
- Blocking buffer. StartingBlock T20 (TBS) Thermo Scientific (Pierce) cat# 37543. When testing for Phospho-proteins it is important to use Tris-based buffers (TBS) instead of phosphate-based buffers (PBS).
- Primary antibody reactive with a single protein (or a group of proteins) and proven to be reactive with tissue lysate samples (OriGene ProteoScan Assay Validation Array PA100001).
- **Secondary Antibody for direct detection** (Fluorescence or Colorimetric)
 - Fluorescently –labeled secondary antibodies (DyLight 649 –conjugated anti-X IgG with minimal cross-reactivity to human serum proteins, Jackson ImmunoResearch Laboratories).
 - Colorimetric: Peroxidase-labeled secondary antibodies (HRP –conjugated anti X IgG with minimal cross-reactivity to human serum proteins, Jackson ImmunoResearch Laboratories).

- **Tyramide Signal Amplification Method (Fluorescence or Colorimetric)**
 - Peroxidase-conjugated secondary Abs (HRP –conjugated anti X IgG with minimal cross-reactivity to human serum proteins, Jackson ImmunoResearch Laboratories).
 - Peroxidase-conjugated Streptavidin (HRP –conjugated Streptavidin, Jackson ImmunoResearch Laboratories) for TSA-biotin colorimetric assays.
 - Fluorescently –labeled Streptavidin (DyLight 649 –conjugated Streptavidin, Jackson ImmunoResearch Laboratories) for TSA-biotin fluorescence assays.
 - Tyramide-Alexa Fluor 647 (Invitrogen (Molecular probes)) for TSA-fluorescence assays.
 - Biotinyl- Tyramide (RENAISSANCE® TSA™ biotin system Perkin-Elmer or CAS Signal amplification system DAKO) for TSA-biotin assays.
 - Peroxidase conjugated Streptavidin (HRP –conjugated Streptavidin, Jackson ImmunoResearch Laboratories) for TSA-biotin colorimetric assays.
 - DAB substrate (PerkinElmer) for colorimetric assays.
- **Suggested sources for TSA Signal Amplification kits**
 - CAS Signal amplification system (Dako)
 - Renaissance TSA biotin system (PerkinElmer)
 - Tyramide Signal Amplification (TSA) kit Invitrogen (Molecular probes)

Equipment and Instruments

- Humidified hybridization chamber (use 150 mm petri-dish (see below) or Chip Clip slide holder with single well incubation chamber (Whatman cat# 10486081 & 10486137))
- Perfect Western 6-sectional short (B130) GenHunter (www.Genhunter.com)
- Array reader and analysis software. For example GenePix 4100 and GenePix pro for fluorescent detection.
- High resolution Scanner (colorimetric assays)
- Analysis software (Microvigene™ software from VigeneTech)

Introduction

Biomarkers are one of the most important tools in the fight against cancer and are the focus of intense research. Cancer biomarkers such as the prostate specific antigen (PSA) are routinely used for early detection. Other markers are used to determine the regimen of treatment and the use of therapeutic agents such as Herceptin (a monoclonal antibody against the HER-2 receptor) in patients with over expressed receptor.

A large number of methods are available for discovery and evaluation of cancer biomarkers, each with its unique advantages. These mainly complementary methods include sequencing, genetic microarrays, northern blots, qPCR, western blots, tissue immunohistochemistry (tissue arrays) and reverse phase protein arrays (tissue lysate arrays). The soaring number of putative genetic targets linked to neoplasia requires high-throughput approaches for discovery and validation.

Reverse Phase Protein Arrays (RPPA) are the major tool for quantitative detection of changes in protein expression profiles and post-translational modifications, such as phosphorylation, associated with cancer. This is the only quantitative method to provide large-scale screening of hundreds to thousands of samples in a single experiment. Proteins that display distinct profiles in cancer tissues are regarded as potential biomarkers for cancer and are of major importance for diagnosis and treatment.

Description of ProteoScan Cancer Lysate Array 1.0

OriGene's ProteoScan Cancer Lysate Array is a high-density large-scale reverse phase protein array assembled from 432 protein lysates of normal and cancer specimens spanning 11 different tissues. Each slide contains 40 high-quality pathology verified samples: 25 cancer samples and 15 normal samples (some tissue types have a lower number of samples). Each lysate is spotted at 4 different concentrations (1,000 µg/ml, 500 µg/ml, 200 µg/ml and 100 µg/ml) in modified RIPA buffer with the intention to minimize the loss of native protein structure. Each sample is spotted in triplicate to ensure accuracy and reproducibility. This design allows for quantitative and semi-quantitative detection of protein expression and modification over a wide range of protein concentrations. With this product researchers can obtain data from 432 samples spanning 11 tissue types in a single experiment. Protein expression can be determined using specific antibodies or probes directed against the target protein. Visualization and quantification of antibody binding is accomplished by using enhanced fluorimetric and colorimetric detection methods or direct fluorescent detection methods.

The ProteoScan Cancer Lysate Array contains 33 sub-arrays each with the configuration of 15X15 spots. Fluorescent positive placement controls (BSA-Cy3, BSA-Cy5 and mixture of the two) and background (negative) controls (buffer only and BSA at 1,000, 500, 200 and 100 µg/ml) are placed in each sub-array. Each third sub-array (last sub-array for each tissue) includes additional controls: IgG mix (human, mouse and rabbit) positive controls at 10-0.156 µg/ml each; purified recombinant β-actin spotted at 10- 0.64 µg/ml; HEK293T lysate and one over-expressed protein lysate from a list of 11 different genes over expressed in HEK293T cells. Array layout, sample location and associated clinical data for each biospecimen can be found at OriGene website and accompanying USB flash drive.

Description of ProteoScan Assay Validation Array 1.0

The ProteoScan Assay Validation Array is offered as a tool for optimizing and validating the methods and reagents that will be used with the ProteoScan Cancer Lysate array. This small array was assembled with 44 protein lysates from normal and cancer specimens spanning 11

different tissues. Each lysate is a mixture of 2-3 different lysates from the same tissue, thus increasing the possibility for positive reactivity. OriGene does not provide the identity of the samples spotted on the Assay Validation Array. The array contains two normal and two cancer samples from each tissue type (total of 44) spotted in triplicate at a single concentration of 1,000 µg/ml in modified RIPA buffer. The design and overall appearance of the Assay Validation Array was kept as close as possible to that of the sub-arrays of the ProteoScan Cancer Lysate array.

The array includes: fluorescent positive placement controls (BSA-Cy3, BSA-Cy5 and mixture of the two), background (negative) controls (buffer only and BSA at 1,000, 500, 200 and 100 µg/ml); IgG mix positive controls (purified human, mouse and rabbit IgG mixed in equal amounts at 10-0.156 µg/ml each); HEK293T lysate and over-expressed protein lysate of TP53 spotted in 4 different dilutions (0.5X-0.004X).

For details on array preparation and quality control, see Appendix A.

Array Layouts

The ProteoScan Cancer Lysate Array contains 33 sub-arrays each with the configuration of 15X15 spots. A sub-array is a group of samples that have been spotted onto the array in a defined pattern. Each tissue type contains 3 sub-arrays and the layout of the sub-arrays is displayed below. Please note that for each tissue the layout (not samples) of the first two sub-arrays (one and two) is identical but the layout of sub-array three differs slightly. Sub-array three contains additional controls that add significant utility to the array (see below for details). An excel spreadsheet containing detailed information on the tumor tissue samples is available for download from the OriGene website and accompanying USB flash drive.

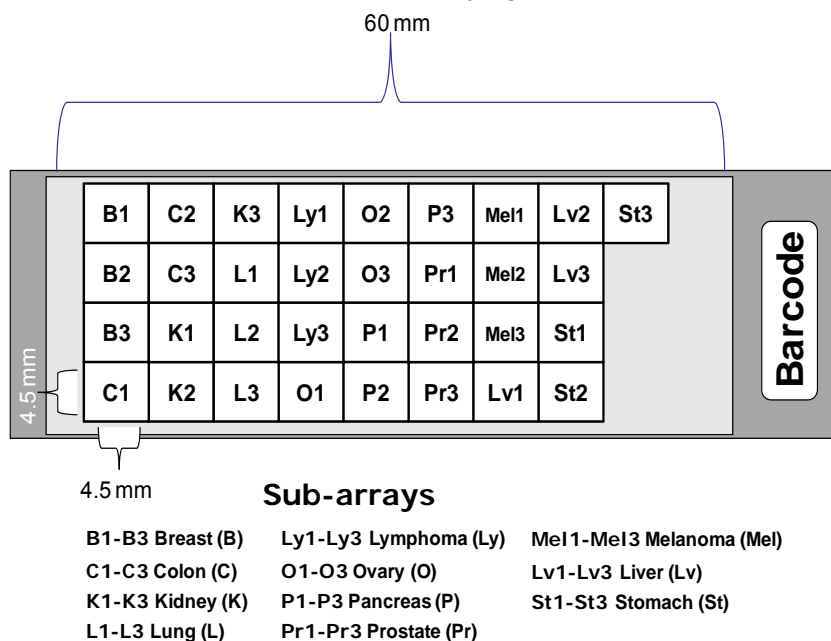


Fig. 1 ProteoScan Cancer Lysate array 1.0

This figure depicts the array layout with cancer type indicated by letter and sub-arrays by number (1-3). Sub-arrays one and two have identical layouts whereas sub-array three includes additional controls and has a slightly different layout. See image below for details.

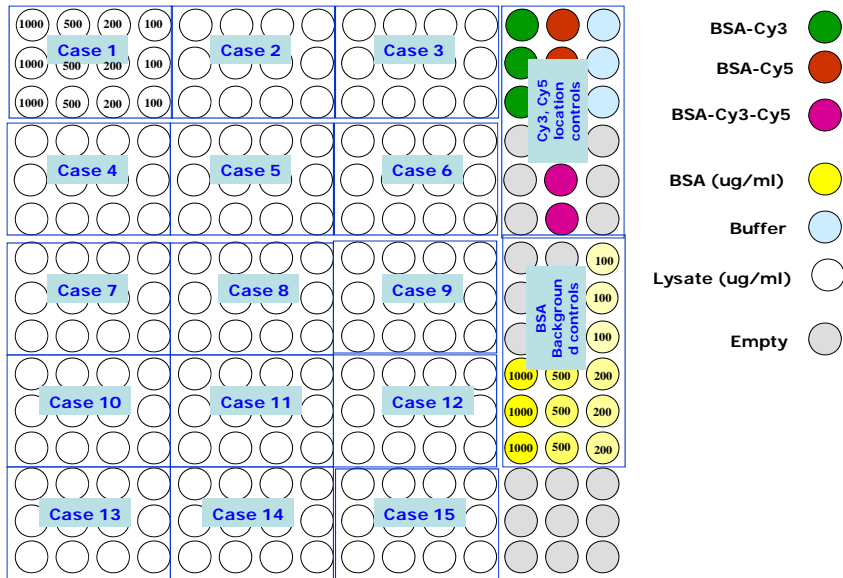


Fig. 2 ProteoScan Cancer Lysate Array sub-array one and two layout.
 Layout of sub-arrays with designations one and two are shown. Numbers and shading are used to indicate amount of protein spotted. The positions of the location controls (Cy-dyes) and background (negative) controls (buffer only and BSA) are noted.

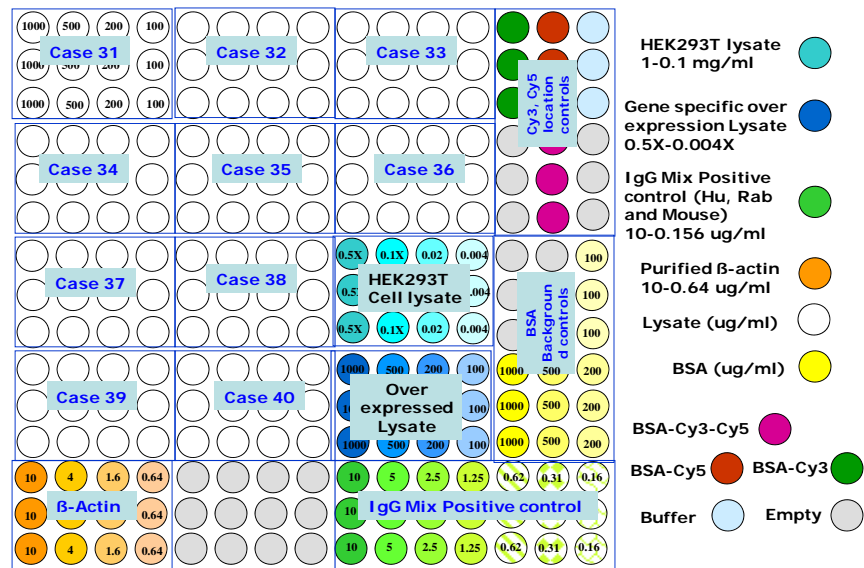


Fig. 3 ProteoScan Cancer Lysate Array sub-array three layout.
 Layout of sub-arrays with designation three are shown. Numbers and shading are used to indicate amount of protein spotted. The positions of the location controls (Cy-dyes), background (negative) controls (buffer only and BSA), β -actin, HEK293T lysates, over-expression lysates and IgG mix positive controls are noted.

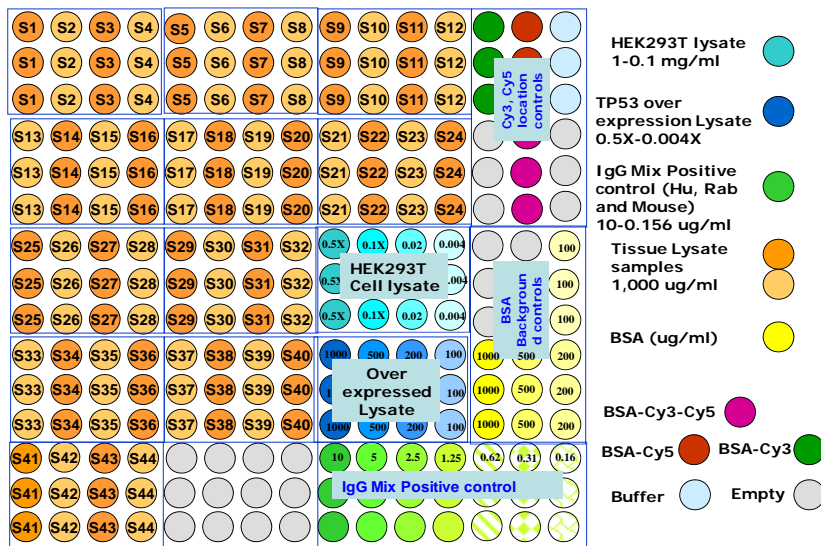


Fig. 4 ProteoScan Assay Validation Array layout.

Layout of Assay Validation array is shown. Numbers and shading are used to indicate amount of protein spotted. The positions of the location controls (Cy-dyes), background (negative) controls (buffer only and BSA), HEK293T lysates, TP53 over-expression lysate and IgG mix positive controls are noted.

Array controls

Each sub-array of the ProteoScan Cancer Lysate Array contains fluorescent positive placement controls (BSA-Cy3, BSA-Cy5 and mixture of the two) and background (negative) controls (buffer only and BSA at 1,000, 500, 200 and 100 µg/ml).

Each third sub-array (last sub-array for each tissue) contains additional controls including: IgG mix control (purified human, mouse and rabbit IgG mixed in equal amounts at 10, 5, 2.5, 1.25, 0.6, 0.3 and 0.15 µg/ml). These positive controls should always give a signal independent from the specific detection method. Additional controls include HEK293T lysate and 11 different proteins over-expression lysates (expressed in HEK293T cells and spotted at 0.5, 0.1, 0.02 and 0.004X dilution of the lysate (1X is about 2 mg/ml for most lysates). HEK293T lysate (without over expressed protein) are spotted at 1,000, 500, 200 and 100 µg/ml).

Another feature of the array is purified β -actin recombinant protein spotted at 10, 4, 1.6 and 0.64 µg/ml. together with the IgG positive control, the β -actin controls provide internal standards that can be used to estimate the amount of target proteins in each sample. BSA spotted at amounts corresponding to the protein concentrations of the tissue lysate samples serves as a control for background. Each sub-array contains one set of background controls that are identified with specific sub-array location allowing local background correction for each sub-array if needed. The quality of each print was verified by Sypro-ruby protein staining and comparing the IgG positive control from all 11 sub-arrays. In general the SD between triplicates is less than 10% (many are around 1%) and the same is true for the IgG positive controls across the array.

Technical Notes

Before you start

Many factors will affect the success of the assay. However, the quality of the primary antibody (Ab) is the single most important factor. Here are some suggestions regarding antibody selection:

- Choose only high quality antibodies from a reliable source. Abs with a proven track record of identifying the target protein from the literature or validated antibodies are the most desirable.
- Test the antibody by Western blotting with extract from a cell line known to express this protein. Test the extract at several concentrations starting at 25-50 μg total protein and 2-3 dilutions with lower protein concentrations.
- The Ab should give a predominant single band. If not, discriminating between positive and false-positive reactivity on the array will be extremely difficult.
- We recommend testing the array at 1:50 to 1:400 Ab dilutions (most at 1:100). A good antibody will give positive Western blot results at dilutions (>2-5X) higher than the one intended for use with the array.
- **For best results: Use OriGene ProteoScan Assay Validation Array SKU PA100001 to pre-qualify your antibody and validate your reagents and procedures.**

The ProteoScan Assay Validation Array is offered as a tool for testing, optimizing and validating the methods and reagents that will be used with the ProteoScan Cancer Lysate Array. The primary difference between the Assay Validation Array and the Cancer Lysate Array is sample identity-all other components of the two arrays are identical. Thus, this is the most cost-effective method for testing and validating the conditions and reagents that will be used with the ProteoScan Cancer Lysate Array, particularly primary antibodies.

The quality of the secondary antibody is also very important and these antibodies should have no cross-reactivity with human proteins. We found that secondary Abs with minimal cross-reactivity to human serum proteins (Jackson ImmunoResearch lab) consistently display minimal reactivity with our samples (range of 1:100-400 dilution). Abs from other companies may also be suitable for use.

It is possible to multiplex the assay using both Cy5 and Cy3 channels. This requires the use of two antibodies from different species and secondary antibodies that are strictly species specific. TSA enhancement assays cannot be multiplexed. It is important to note that the Cy5 channel provides much higher sensitivity than the Cy3 one. Thus, we recommend using the Cy3 channel only for highly sensitive antibodies and for more abundant protein targets such as housekeeping genes (β -actin and others).

Fluorescently-labeled Abs provide the most direct quantitative results and show good sensitivity. When necessary (often), the signals can be enhanced using a Tyramide Signal Amplification (TSA) method to enhance the assay signal. Methods that use amplification protocols are generally more sensitive but often provide less quantitative results than direct detection methods. We found the Biotin-TSA enhancement method to provide the highest sensitivity particularly when used in conjunction with colorimetric detection. However, additional optimization of the assay is usually required for these methods.

When using biotin-avidin detection systems, care should be taken to avoid reactivity with endogenous biotin or biotin found in some blocking buffers (such as milk). Care should be taken to avoid reactivity of endogenous peroxidase with TSA enhancement methods

Finding the optimal solution for blocking non-specific sites without compromising sensitivity is also very important. Avoid phosphate-based buffers if testing anti phospho-protein antibodies. We routinely use the StartingBlock TBS-tween20 (Pierce) as blocking buffer.

Experimental Procedures:

Notes: Most incubations and washings are performed with the Perfect Western 6-sectional short (B130) slide tray GenHunter (www.Genhunter.com) with the nitrocellulose side facing up. All incubations are at room temperature unless stated otherwise.

Do not touch the nitrocellulose part of the array as it will damage the print area.

Reagents:

- Washing buffer TBST. (20 mM Tris, 150 mM NaCl, 0.05-0.1% Tween20, pH 7.5).
- Blocking buffer. StartingBlock T20 (TBS) Thermo Scientific (Pierce) cat# 37543. Alternative: 5% Skimmed milk in TBS-T can also be used as blocking buffer.
- 30% H₂O₂ (TSA assays).
- Tyramide amplification buffer (TSA assays).
- DAB substrate (colorimetric assays).
- DAB buffer (colorimetric assays).

General Protocol

Note: Protect the slide from excessive exposure to light. Cover with aluminum foil during incubations.

1. Remove the array from storage and hydrate each array with 10 ml of water for 45 min.
2. Equilibrate the array with 5 ml of TBS-T for 5 min.
3. Block for 60 min at RT with rotation using 10 ml of blocking buffer (StartingBlock TBS-tween20 or your choice of blocking buffer).
4. Prepare primary antibody at the appropriate dilution in blocking buffer (arrays are tested at relative low Ab dilutions 1:50-200).

Note: When testing two antibodies simultaneously add both Abs to the same incubation.

5. Incubate with primary antibody. Use 2 ml solution for incubation in the Perfect Western slide tray (preferred). Incubate overnight with rotation at 5°C.

- **Low volume (450-700 µl) alternative.**

- a. Use the Chip clip slide holder with single well incubation chamber (Whatman) at 500-700 µl. Cover and incubate overnight at 5°C with shaking.
 - b. Place a parafilm patch on the bottom of a humidified Petri-dish (attach a folded piece of wet paper to the top cover) and prepare 500 µl Ab sample. Pipette 450 µl onto the parafilm patch (no bubbles) then place the array face down on the solution ensuring that there are no air bubbles and the entire array is in contact with the liquid. Wrap with Saran wrap (optional) and incubate overnight at 5°C without shaking.
 1. Remove slide and adsorb the liquid onto a Kimwipe (do not touch the printed area).
 2. Move to the Perfect Western slide tray.
- 6) Do a quick wash with 10 ml TBST.
 - 7) Wash twice with 5 ml of blocking buffer 5 min each.
 - 8) Wash twice with 5 ml of TBST 5 min each.

Detection Methods

Direct detection (non-enhanced method)

1. Add 2.5 ml of secondary Ab in blocking buffer.
 - **Fluorescence:** add fluorescently-labeled secondary Ab in blocking buffer to the slide (face up). We recommend using the Dylight 649 Goat anti X IgG (Jackson ImmunoResearch at 1:400 dilution). For detecting two antibodies simultaneously using both Cy5 and Cy3 channels add both secondary Abs (Dylight 649 Goat anti X IgG and Dylight 549 Goat anti Y IgG) to the same incubation.
 - **Colorimetric:** add peroxidase-labeled secondary Ab in blocking buffer. We recommend using the HRP-conjugated Goat anti X IgG (Jackson ImmunoResearch at 1:1,000 dilution).
2. Incubate 60 min RT with rocking
3. Aspirate liquid and do a quick wash with 10 ml TBST.
4. Wash once with 5 ml of blocking buffer 5 min.
5. Wash 3 times with 5 ml of TBST 5 min each.
 - **For colorimetric assays:** Add 10 ml of DAB substrate solution. Incubate 1-10 min or as needed until color develops.
6. Wash 2-3 times with 10 ml of water.
7. Remove from tray and air dry (covered).
8. Read slide using appropriate Scanner and analysis software.

Tyramide Signal Amplification (TSA) enhancement protocols

Fluorescence (Alexa Flour 647 dye TSA)

1. Follow steps 1-8 of general protocol.
 2. Add 2.5 ml of HRP- conjugated Goat anti-X IgG (min X-reactivity Jackson ImmunoResearch 115-035-062) diluted 1:1,000 in blocking buffer.
 3. Incubate 60 min at RT with rocking.
 4. Aspirate liquid and do a quick wash with 10 ml TBST.
 5. Wash once with 5 ml of blocking buffer 5 min.
 6. Wash 3 times with 5 ml of TBST 5 min each.
 7. Prepare the Alexa Flour 647 dye TSA enhancement kit (Invitrogen) according to the manufacturer's instructions.
 - a) Prepare the tyramide stock solution by dissolving the material in 150 μ l of DMSO.
 - b) Prepare the tyramide working dilution (500 μ l) by diluting the tyramide stock solution 1:50 in amplification buffer.
 - c) Prepare hydrogen peroxide by diluting 1 μ l of 30% H_2O_2 in 200 μ l of amplification buffer.
 - d) Add 5 μ l of the diluted H_2O_2 (1:100) to the 500 μ l tyramide working dilution.
 8. Incubate with the array slide for 3-10 minutes using the low volume procedure (#5).
 9. Move to the Perfect Western dish and do a quick wash with 10 ml TBST
 10. Wash three times with 5 ml of TBST 5 min each.
 11. Wash 2-3 times with 10 ml of water.
 12. Air dry and read using the appropriate fluorescence scanner and analysis software.
- You can also use the procedure described below for enhanced colorimetric assay replacing the HRP-conjugated Streptavidin steps with Dylight 649 Streptavidin (Jackson ImmunoResearch) diluted 1:1,000.

Enhanced colorimetric assay

1. Follow steps 1-8 from general protocol.
2. Add 2.5 ml of HRP- conjugated Goat anti-X IgG (min X-reactivity Jackson ImmunoResearch 115-035-062) diluted 1:2,500 in blocking buffer (dilution range is 1,000-5,000).
3. Incubate 60 min at RT with rocking.
4. Aspirate liquid and wash as in steps 4-6 of the TSA fluorescence assay.
5. Prepare the Biotinyl Tyramide reagent according to the manufacturer's instructions (Renaissance TSA biotin system, Perkin Elmer).
 - a) Prepare the tyramide working dilution (500 μ l) by diluting the tyramide stock solution 1:50 in amplification buffer.
 - b) Prepare hydrogen peroxide by diluting 1 μ l of 30% H₂O₂ in 200 μ l of amplification buffer.
 - c) Add 5 μ l of the diluted H₂O₂ (1:100) to the 500 μ l tyramide working dilution.
6. Incubate with the array slide for 3-10 minutes using the low volume procedure (#5).
7. Move to the Perfect Western dish and do a quick wash with 10 ml TBST
8. Wash three times with 5 ml of TBST 5 min each.
9. Add 2.5 ml of HRP- conjugated Streptavidin (Jackson ImmunoResearch) diluted 1:2,500 in blocking buffer (dilution range is 1,000-5,000).
10. Incubate 30 min at RT with rocking.
11. Aspirate liquid and wash as in steps 4-6 of the TSA fluorescence assay.
12. Add 10 ml of DAB substrate solution. Incubate 1-10 min or as needed until color develops.
13. Wash 2-3 times with 10 ml of water.
14. Air dry and read using the appropriate high-resolution scanner and analysis software.

Data Analysis

Note: Use the ProteoScan Cancer Lysate galfile.gal file available on OriGene website to associate each spot with the appropriate sample.

Data analysis and statistical analysis are important aspects of reverse-phase protein arrays. A variety of programs and packages are available for analysis of the data and each researcher should choose the most appropriate methods for their assay. A RPPA-specific module for the Microvigene™ software from VigeneTech is available and currently used by several of the laboratories that pioneered RPPA arrays. This software uses curve fitting models (linear or non-linear (supercurve) to provide curve quantification, such as Y0. Y0 is the measurement of curve in the Y direction or intensity values, representing the level of target protein expression in each sample. Either the IgG positive control standard curve or the recombinant β -actin standard curve permits approximation of target protein concentration.

OriGene offers a very simple calculation that permits the identification of cancer samples that show aberrant expression (high or low) of the tested protein relative to normal samples. We calculate the cancer expression index by dividing the readout from each sample with the median expression from the normal samples of the same tissue. This data can be represented in a heat map style using conditional formatting option in Microsoft office Excel (as appears on our web). You can do that using the Y0 values giving one column format or simply use the values from each of the four concentrations giving a four columns format. To do that copy your data to the appropriate columns of the heat map formatting file. You can use either the slope values (Y0) in one column and use the other for independent repetition of the assay or copy the values of each of the four protein concentrations to the appropriate columns. Please note that for tissues with less than 40 samples an empty space was introduced to keep the same appearance for all tissues. The heat map representation permits immediate identification of cancer samples that

have aberrant expression profiles. We find a good correlation between our simple heat map analysis and the Microvigene method, please note that this simple analysis method is much less sensitive than the Microvigene method (see above) and frequently samples with low signals need to be discarded from the analysis.

Further statistical analysis can be performed using the data provided with each sample and a suitable statistical analysis package.

Appendix A

Quality of biospecimens

All lysates are derived from high-quality biospecimens and include substantial clinical data (Available on our website). The Cytomyx tissue biorepository contains over 130,000 high-quality human biospecimens representing over 12,000 donor cases, all collected from accredited academic medical institutions in the United States. Samples and data were collected under the highest bioethical principles using IRB-approved protocols, HIPAA guidelines and donor informed consents. The informed consents included specific language regarding use of clinical materials and information with genetic research specifically mentioned. The collection and storage standard operation procedures are compliant with all existing federal, state, local and institutional requirements.

All tissues were processed within 30 minutes of ischemia. Tissue procurement and clinical data collection utilized the BGR® (Biomaterials and Information for Genomic Research) system to ensure data integrity for each sample. Pathology diagnosis with histological subtype; tissue of origin and site of finding; AJCC TNM staging and grading; diagnostic tests performed at the medical center; and sample composition (%tumor, %normal, %stroma, %necrosis) as recorded by a board-certified pathologist while reviewing an H&E slide of the sample.

Protein Extraction

Tissues were removed from the protective OCT by incubation in TBS buffer containing protease inhibitor cocktail (sigma P2714). The samples were homogenized and extracted in modified RIPA buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% NP40, 0.25% Deoxycholate) containing protease inhibitor cocktail (sigma P2714), 0.4 mM PefaBlock SC plus (Roche 11873601001) and PhosStop phosphatase inhibitor cocktail (Roche 04906845001).

Following extraction the lysates were spun down to remove non-soluble material and stored at -80°C. Protein content was determined by BCA assay. All lysates were adjusted to 1 mg/ml using modified RIPA buffer and diluted to 500, 200 and 100 µg /ml in reduced detergent RIPA buffer.

Array Printing

The array was printed on Schott single pad nitrocellulose slides using 110 µM pins (Aushon Biosystems) and contains 33 sub-arrays of 15X15 spots. Each tissue type contains 3 sub-arrays with samples printed in triplicate. The amount of deposit is approximately 300 pl. The quality of each printing was verified by Sypro-ruby protein staining using the IgG positive control from all 11 sub-arrays. The standard deviation (SD) among triplicates is less than 10% (usually around 1%).

Frequently Asked Questions

Q: What is the ProteoScan Cancer Lysate Array?

A: The ProteoScan Cancer Lysate Array is an array of 432 protein extracts from cancer and normal tissues, normalized by total protein concentration and spotted onto Nitro-cellulose slides.

Q: How many samples and cancer types are represented on the array?

A: There are 432 samples from 11 types of tissue. Each tissue is represented by 40 samples; 25 samples are from cancer tissues and 15 are from normal tissues. Some tissues have fewer samples due to limited availability (Prostate 13 normal; Melanoma 22 cancer and 14 normal; Liver 23 cancer). There are four different protein concentrations for each sample and these are spotted in triplicate.

Q: How were these tissue samples obtained?

A: Biospecimens were obtained from accredited academic medical institutions in the United States. Samples and data were collected under the highest bioethical principles using IRB and full adherence to HIPAA guidelines.

Q: What clinical data accompany each sample?

A: Samples come with detailed pathology reports AJCC TNM staging and grading; diagnostic tests performed at the medical center; and sample composition (%tumor, %normal, %stroma, %necrosis) as recorded by a board-certified pathologist while reviewing an H&E slide of the sample. Data is coded to protect donor identities.

Q: I purchased both the ProteoScan Cancer Lysate Array and the ProteoScan Assay Validation Array. How can I distinguish between the two?

A: There are several ways to determine which slide belongs to which array: First, the protective container contains only slides from one type of array and it is clearly labeled. Second, The ProteoScan Assay Validation Array is specifically labeled with the product name. Third, the nitrocellulose dimensions of the validation slide are smaller (51mm length) than those of the ProteoScan Cancer Lysate slide (60 mm). Fourth, Scan for Cy3 or Cy5 and determine the slide layout based on the BSA-cy3 or BSA-cy5 number and location. If you are still unable to distinguish between them call OriGene technical support for help.

Q: Was laser capture micro-dissection or any other micro-dissection procedure performed to separate tumor cells from non-tumor cells?

A: No, the tissue was not micro-dissected to separate tumor from non-tumor cells. The selected samples have high tumor content as determined by OriGene's pathologist. The specific composition of each sample is available on the datasheet from OriGene's website.

Q: Were any preservatives or embedding agents used?

A: The tissue samples are frozen in OCT, which serves as an excellent protectant against the effects of long term -80°C storage. The OCT is removed prior to processing the tissue for protein extraction.

Q: Are the proteins in the array denatured?

A: The extraction is performed without denaturing agents such as SDS, urea etc. We used only mild non-denaturing detergents (NP40 and DOC) to preserve the native structure of soluble proteins as much as possible.

Q: Why do you add PhosStop to the extracted samples?

A: The phosphorylation state of many proteins is significantly altered in many cancers. The addition of PhosStop phosphatase inhibitor cocktail preserves these important modifications for detection using phospho-specific antibodies.

Q: What changes can be detected using the ProteoScan Cancer Lysate Array?

A: The array can be used to detect quantitative changes in protein expression profiles, post-translational modifications such as phosphorylation and other changes provided you have a suitable specific antibody.

Q: Why are there four different protein concentrations on the array?

A: The spotted sample includes abundant and less-abundant proteins. To obtain quantitative or semi-quantitative data it is important to cover a large dynamic range. Often, the slope of the Ab response over the 4 dilutions can be correlated with the amount of that particular protein.

Q Were any of the normal and cancer samples taken from the same donors?

A: Some samples are paired but the majority are not. These paired samples can be identified by the identical pathology report numbers listed for different samples.

Q: What methods of detection can we use?

A: In principal you can use colorimetric, fluorescence and chemiluminescent methods for detection. Certain methods may require the use of special equipment (reader) and the development of an enhancement protocol to increase sensitivity. We routinely use enhanced (TSA) fluorescence and colorimetric methods for detection.

Q: Can we purchase individual protein lysates from specific samples included in the array?

A: Yes, we maintain a stock of protein lysates for most samples. You can purchase these samples directly from OriGene by giving the case number.

Q: How can we normalize the results to the relative amount of cell extract spotted?

A: There is no perfect solution for this problem. We pre-normalized the array based on total protein in each sample. You can use the expression results from one or more housekeeping proteins such as β -actin, GAPDH, α and β tubulin etc. However, the expression of these genes can vary among different tissues and also display differences between tumor and normal tissues. In fact, we detected such differences using β -actin as a probe. These possibilities should be taken into account when analyzing the data

Q: Why should I use the ProteoScan Assay Validation Array?

A: Before testing the ProteoScan Cancer Lysate Array it is vital to validate the primary and secondary antibodies, identify the appropriate usage dilution and optimize the experimental procedure. The primary difference between the Assay Validation array and the ProteoScan Cancer Lysate array is sample identity-all other components of the two arrays are identical. Thus, this is the most cost-effective method for testing conditions and reagents that will be used with the ProteoScan Cancer Lysate array.

Q: Why are the Assay validation slides not included with the ProteoScan Cancer Lysate array?

A: We prefer to give our customers the freedom to choose according to their needs. While we believe that using our validation array for optimization is the best option not all scientists will

require this step. By providing the ProteoScan Cancer Lysate and ProteoScan Assay Validation arrays as separate items, OriGene is able to minimize the cost of the product for all customers.

Q: Why is the identity of the samples in the Assay Validation Array not disclosed?

A: The ProteoScan Assay Validation Array is a supporting tool to help customers achieve success with the ProteoScan Cancer Lysate Array. Many of the samples are a mixture of several lysates from the same tissue type and thus are not representative of a specific disease stage. Because the samples are pooled, it is not possible to accurately link them to clinical data as with the ProteoScan Cancer Lysate array

Q: I have an Ab that is shown by the manufacturer to be positive by Western blot. Is it necessary to test it with the ProteoScan Assay Validation Array?

A: We highly recommend that you do. We have tested several antibodies shown by the provider to be highly reactive by Western blot that completely failed to give any response with the array or even with lysate from cell lines. Using the ProteoScan Assay Validation Array can save you the expense, frustration and time associated with such reagents.

Q: My data indicate a high level of background. What can I do to reduce this?

A: There are several possible causes. The most likely cause of high background is using too high of a concentration of antibody for probing. We suggest diluting the primary antibody and screening with OriGene Assay Validation Array to determine the optimal antibody dilution factor. Higher background can also be attributed to secondary antibodies. Make sure you use qualified secondary antibodies and use appropriate dilution. If TSA enhancement with Biotin-tyramide was used, we recommend extending the blocking time or changing the blocking buffer so that the signal from endogenous biotin is minimized. High background can also come from endogenous peroxidase. Quenching endogenous peroxidase can be achieved by treating the sample with 3% H₂O₂ in PBS for 10 minutes.

Q: I didn't get any signal from any of the samples or the controls.

A: The IgG control will almost always give a positive signal if the procedure was executed properly. Most likely, an essential component was not added or a step was missed.

Q: The signal from the experimental samples is very low but the signal from the controls is very good.

A: It is likely that the primary antibody is not sufficiently sensitive for use on the array or that the correct dilution factor was not used. We suggest further testing the primary antibody by screening with OriGene Assay Validation Array to determine the optimal antibody dilution factor.

Q: After developing the array, a streak that looks like a comet has appeared.

A: Unless this streak covers a spot, it will not affect the reading of the actual data from the array. If it does cross a spot, it will be necessary to remove that data point from the analyses. There are two possible causes for this; one, the array became dehydrated at some step during the screening process. It is essential to keep the array hydrated until the final drying step. Alternatively, the solutions used for screening may not have been thoroughly mixed before they were added to the array.