

cell signaling

Panorama™ Ab Microarray Cell Signaling Kit: A Unique Tool for Protein Expression Analysis

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Application Notes

- Easy and fast protocol
- Convenient Cy3/Cy5 microarray format
- Superb spot morphology for reliable data analysis
- No expensive or unusual equipment

Introduction

There is a growing need for technologies that allow global molecular characterization of biological samples. The ability to identify multiple proteins simultaneously has many applications in basic biological research as well as in disease diagnosis and treatment. The use of DNA arrays for profiling mRNA expression in cells has provided valuable information in many biological areas. However, there is not always a direct correlation between the mRNA level and the expression of the protein. Therefore, a method that can assay proteins is required for meaningful analysis. Whereas DNA/RNA/oligo arrays give information on the genetic defects that may cause disease, protein microarrays provide precious information about corresponding functional states. Sigma-Aldrich's new Panorama™ Ab Microarray Cell Signaling Kit (Product Code [CSAA1](#)) is the first in a line of antibody-based microarrays developed to provide such a solution and can be used to profile expression of proteins in samples.

Microarray format and antibody content

Panorama Ab Microarray Cell Signaling Kit contains 224 different antibodies each spotted in duplicate in 4 x 8 grids on nitrocellulose-coated glass slides. Each grid contains 7 antibody duplicates plus a Cy[™]3- and Cy[™]5-conjugated BSA positive control and a non-labeled BSA negative control resulting in a total of 512 spots (Figure 1).

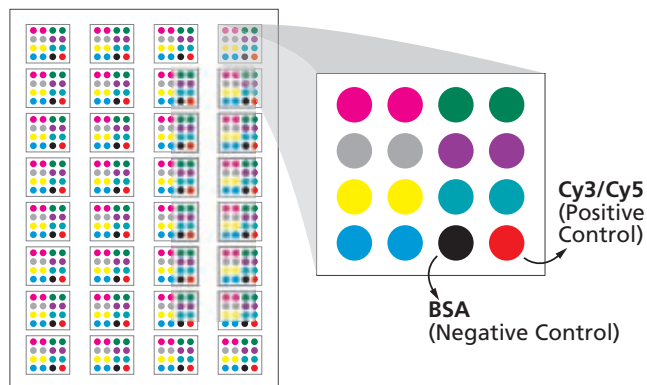


Figure 1. Spotting format.

Each spotted antibody in the array has been validated for its ability to bind proteins in the array assay using samples from human, mouse and rat; some are phospho-specific for their targeted protein (FAK, MAPK, Raf, p38, Pyk2, PAK1, and DAPK).

The antibodies cover biological pathways such as apoptosis, cell cycle, nuclear proteins, neurobiology and phosphorylation. In addition cytoskeleton antibodies (to actins, myosins, and tubulins) are included for normalization purposes, as it is assumed that the expression of these housekeeping proteins does not change with different treatments of sample and control. These antibodies have been carefully selected from the range of over 3,000 antibodies available from Sigma-Aldrich.

Labeling and detection chemistry

Multiple proteins can be simultaneously measured in complex mixtures, using the robust method of direct sample labeling with fluorophores. This method avoids cross-specificity of antibody pairs often seen in classic sandwich assays. Proteins from test and control samples are labeled with the Cy3 or Cy5 monofunctional reactive dyes, respectively. The non-conjugated free dye is removed from the labeled sample by applying the sample on SigmaSpin™ Post-Reaction Clean-Up Columns (Product Code [S_0185](#)). The labeled protein samples (test and control) are mixed and incubated on the microarray. After specific proteins bind to antibodies on the array, the slides are read for differential analysis using standard microarray scanning devices. The total assay takes about 5 to 6 hours.

For details of the protocol, please refer to the technical bulletin at sigma-aldrich.com/antibodyarray.

Background reduction

Obtaining a high signal-to-noise ratio is an important requirement for a meaningful analysis. In the antibody array assay two factors can contribute to background noise: nitrocellulose, which is known to have fluorescent background, and the non-specific binding of proteins in the labeled sample. We have developed a proprietary blocking buffer that reduces most of the non-specific background in the assay (Figure 2). This background reduction permits realization of the full potential of the direct labeling method using fluorescent dyes.

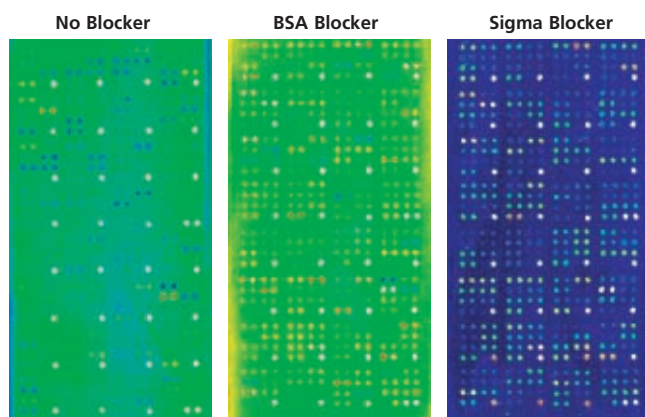


Figure 2. Cy3-labeled NIH-3T3 cell extracts were applied on slides treated by different blocking solutions after spotting.

Specificity, sensitivity and reproducibility

To determine specificity and sensitivity, purified Caspase 9 has been labeled and incubated on the microarray at different concentrations ranging from 3 ng/ml to 100 ng/ml (Figure 3). Specific binding of Caspase 9 to its spotted antibody was observed. The sensitivity was at least 3 ng/ml. It is important to stress that for other antibodies, sensitivity levels will depend on the binding and dissociation constants of the antibody-antigen complexes. Therefore, for other antigens a lower or higher sensitivity may be observed. The variance of signals detected from duplicate spots within the same slide is less than 8%, whereas the variance of those signals detected from spots between slides is 10% (data not shown).

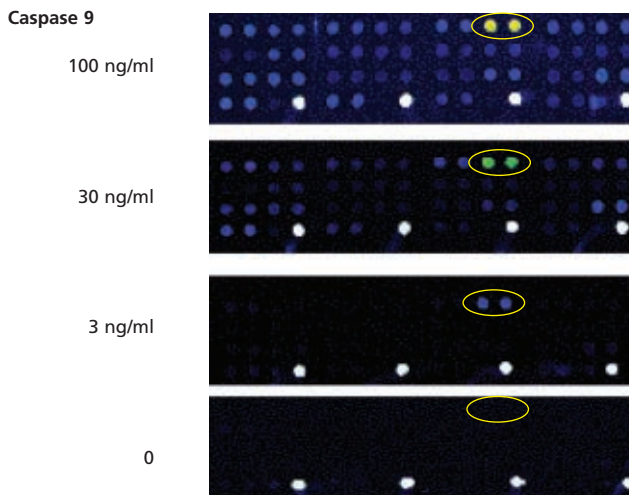


Figure 3. Cy3-labeled recombinant Caspase 9 was applied on array slides at different concentrations. The position on anti-Caspase 9 antibody on the array is circled.

Differential expression of proteins in tissues

The Panorama Ab Microarray Cell Signaling Kit was used to profile the expression of proteins in several mouse tissues. Proteins were extracted from mouse liver, lung, and cerebellum, labeled with Cy3 and applied on the array. As expected, each tissue showed a different pattern of protein expression (Figure 4).

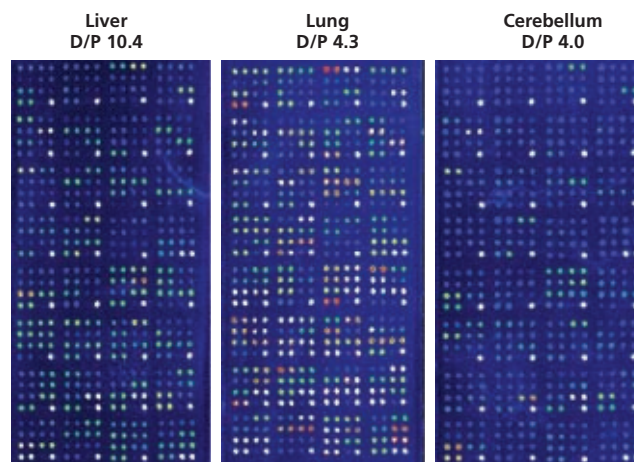


Figure 4. Differential protein expression in mouse tissues.

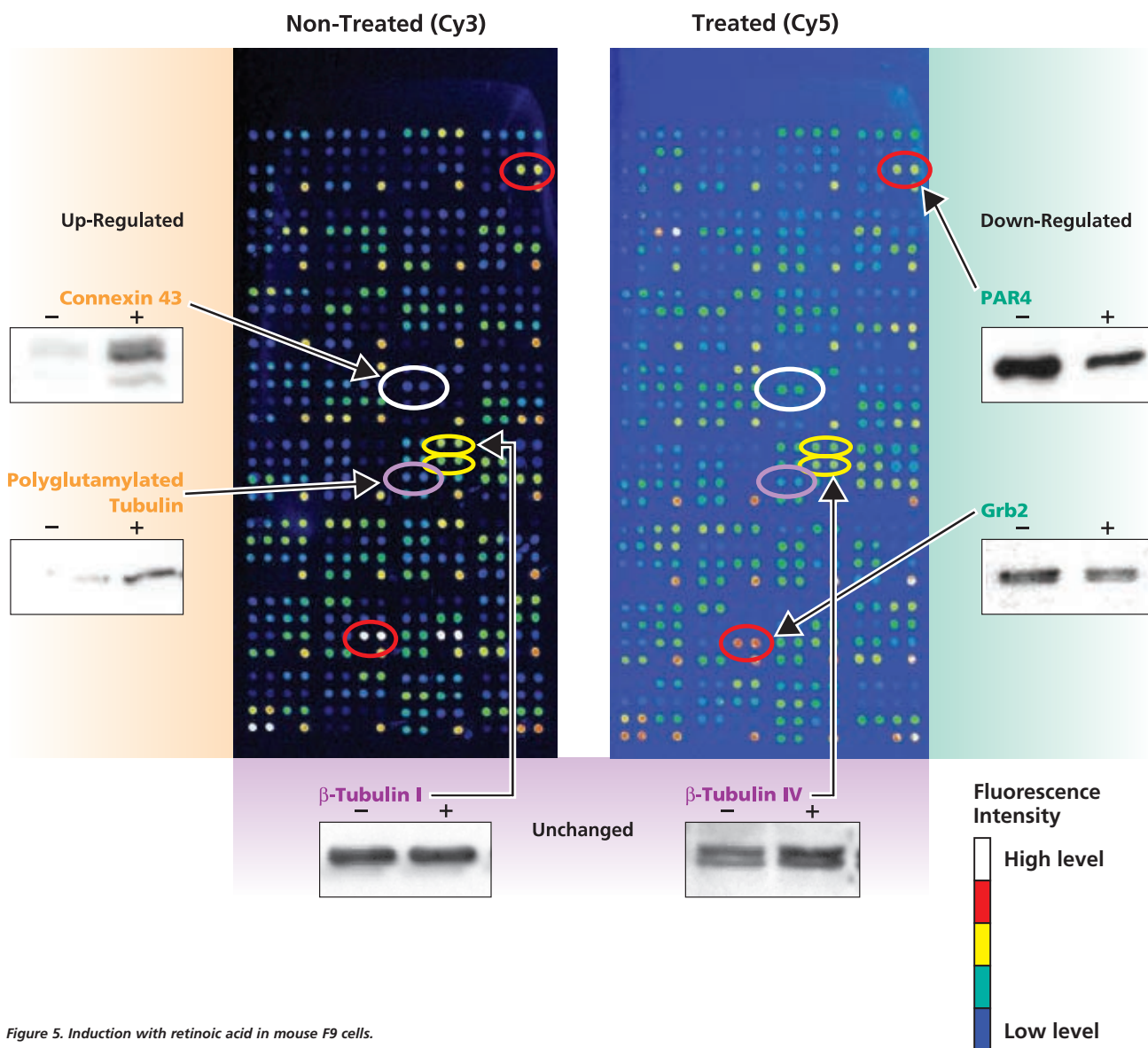


Figure 5. Induction with retinoic acid in mouse F9 cells.

To demonstrate the utility of the kit in monitoring changes in protein expression pattern, mouse F9 stem cells were induced by all-*trans*-retinoic acid. F9 cells were treated for 96 hours with all-*trans*-retinoic acid (10^{-7} M). Extracts were prepared from untreated and treated cells using the Extraction/Labeling Buffer provided in the kit, and labeled with Cy3 and Cy5, respectively. A mixture containing equal amounts of each labeled extract ($5 \mu\text{g}/\text{ml}$) was incubated on the array as described in the kit protocol. The results obtained are illustrated in Figure 5, which show the same slide at the two fluorescence emission wavelengths for

Cy3 and Cy5. Note that the blue background for Cy5 represents the normal and unavoidable background of the nitrocellulose membrane. Proteins that showed changes in protein expression were further analyzed by immunoblotting to confirm the results. These results are summarized in Figure 5. Equal amounts of protein extract ($20 \mu\text{g}/\text{lane}$) from treated and untreated cells were separated by SDS-PAGE and blotted onto nitrocellulose membranes. The proteins were probed with the monoclonal or polyclonal antibodies corresponding to the array and visualized using chemiluminescence.

Summary

Protein arrays are a promising new tool for mass analysis of protein level changes in cells responding to different stimuli. Sigma-Aldrich has developed The Panorama™ Ab Microarray Cell Signaling, our novel antibody array system that contains 224 different antibodies. The kit allows for profiling of hundreds of proteins at the same time. The antibody array is spotted on nitrocellulose-coated slides that can detect protein levels as low as a few nanograms per ml. The antibodies spotted are specific for proteins important in various areas of cell signaling. The Panorama Ab Microarray Cell Signaling Kit provides all the necessary buffers, inhibitors, and labware necessary for extraction and labeling. Whether you're an experienced DNA microarray user and ready to take the next step or are new to microarrays and eager to jump right into protein profiling, the Panorama™ Ab Microarray Cell Signaling Kit is the right tool for you.

Ordering Information

Product	Description	Unit
CSAA1	Panorama Ab Microarray — Cell Signaling Kit	1 kit

Kit Contents

Product Description

Panorama Antibody Slides — Cell Signaling (2 Each)
QuadriPERM Cell Culture Vessel (2 Each)
Extraction/Labeling Buffer
Protease Inhibitor Cocktail
Phosphatase Inhibitor Cocktail I
Phosphatase Inhibitor Cocktail II
Benzonase, Ultrapure
Array Incubation Buffer
Phosphate Buffered Saline, pH 7.4, with Tween 20 (Washing Buffer)
SigmaSpin™ Post-Reaction Clean-Up Columns
Collection Tubes, Polypropylene, 2 ml
Panorama Antibody List — Cell Signaling

For more information and a complete antibody list for our Panorama Ab Microarray Cell Signaling Kit, refer to sigma-aldrich.com/antibodyarray.



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