

# Expression Profiling

## Panorama® Antibody Array Technology

The Panorama Antibody Microarrays are designed for studying protein expression in serum samples, or cell or tissue extracts. The ability to identify multiple proteins simultaneously allows global molecular characterization of biological samples with applications in fundamental cell biology research as well as for disease diagnosis and treatment. The use of DNA arrays for profiling mRNA expression in cells has accelerated research for understanding biological systems from a genomic perspective. However, mRNA undergoes a number of alternative processing steps prior to and following translation. There is often poor correlation between mRNA and protein expression<sup>1</sup> so a method that can assay proteins directly is required for analyzing biologically-relevant events.

With the sensitivity of antibody detection, Panorama Antibody Array technology enables researchers to screen expression changes in large numbers of proteins in significantly less time than it would take using conventional protein detection methodology. Examples where Panorama Antibody Microarrays have been used for such applications include the differentiation of F9 cells,<sup>2</sup> breast cancer samples,<sup>3</sup> and in the maintenance of human embryonic stem cell pluripotency and viability.<sup>4</sup>

The expression of a protein in a cell extract is detected when it binds to its corresponding antibody spotted on the slide. This binding is visualized by a sensitive fluorescent signal created by directly labeling the proteins in the cell or nuclear extract with a fluorescent dye.

### Features and Benefits

- Rapid, multiplex analysis of protein expression
- Use serum, cell or tissue samples
- Antibodies react with human, mouse, and rat
- Compatible with Cy<sup>TM</sup>3/Cy5 detection

### Applications

- Biomarker discovery and drug development
- Identify gene silencing (RNAi) off-target effects
- Cell response to external stimuli (e.g., starvation, growth factors, serum, drugs).
- Differential protein expression in tissue samples (e.g., heart, brain, lung etc.)

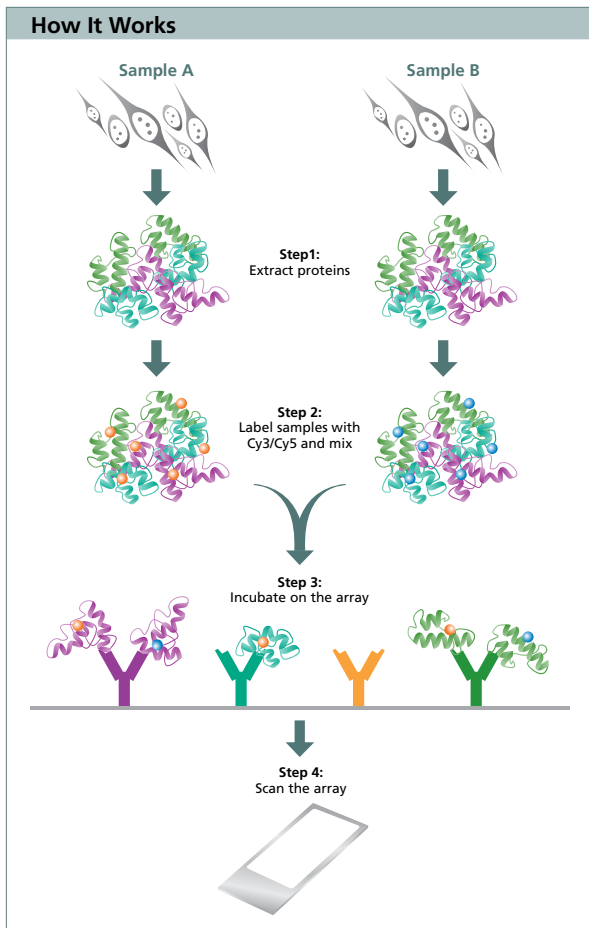
### References

1. Gygi, S.P., et al., *Mol. Cell Biol.*, **19**, 1720-1730 (1999).
2. Kopf, E., et al., *Proteomics*, **5**, 2412-2416 (2005).
3. Celis, J.E., et al., *FEBS J.*, **272**, 2-15 (2005).
4. Armstrong, L., et al., *Human Molecular Genetics*, **15**, 1894-1913 (2006).

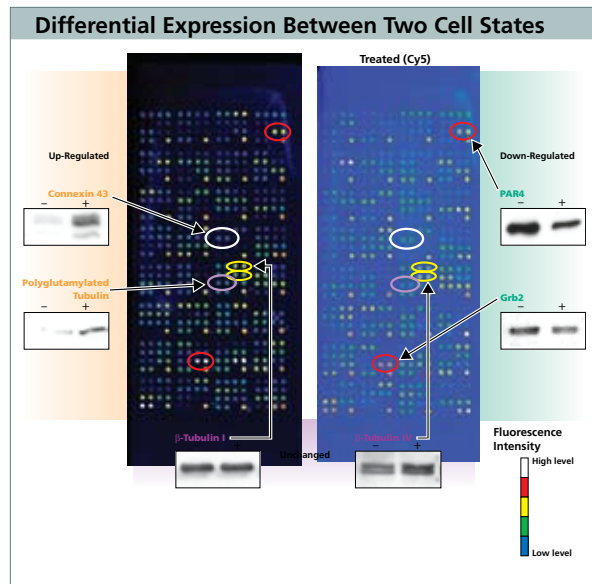


### Components

Panorama Antibody Slides (2 each)
quadriPERM® Cell Culture Vessels (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



The two protein extracts are labeled separately with either Cy3 or Cy5. After the removal of excess label, the labeled protein extracts are combined and incubated with the array. Unbound protein is removed with a brief wash and the array is scanned with any standard fluorescence scanner. The time required to perform the entire procedure (except data analysis) is only 4-5 hours.



Expression of proteins in F9 mouse stem cells induced to differentiate by retinoic acid. The total cell extract of F9 cells non-treated or treated for 4 days with retinoic acid were prepared and labeled with Cy3 and Cy5, respectively. Equal amounts of Cy3 or Cy5 labeled protein were incubated on the slide. The experiment was repeated at least three times.

Using the Panorama Antibody Microarray – Cell Signaling Kit, proteins from mouse F9 cells are observed to be up-regulated or down-regulated in expected fashion following stimulation with 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 Extraction/Labeling Buffer and labeled with Cy<sup>™</sup>3 and Cy5, respectively. A mixture containing equal amounts of each labeled extract (5  $\mu$ g/ml) was incubated on the array as described in the kit protocol. The same slide is shown at the two fluorescence emission wavelengths for Cy3 and Cy5. Changes in expression level were confirmed by immunoblot.

### Publications

Links to abstracts can be found at: [sigma.com/arrays](http://sigma.com/arrays)

Armstrong, L. et al. (2006) "The role of PI3K/AKT, MAPK/ERK and NF signaling in the maintenance of human embryonic stem cell pluripotency and viability highlighted by transcriptional profiling and functional analysis." *Human Molecular Genetics*, Vol. 15, No. 11, 1894-1913.

Celis JE. et al. (2005) "Identification of Extracellular and Intracellular Signaling Components of the Mammary Adipose Tissue and Its Interstitial Fluid in High Risk Breast Cancer Patients." *Molecular & Cellular Proteomics*, Vol. 4, 492-522.

Celis, J. et al. (2005) "Towards discovery-driven translational research in breast cancer." *FEBS Journal*, Vol. 272, 2-15.

Kopf, E. et al. (2005) "Panorama Ab Microarray Cell Signaling Kit: A unique tool for protein expression analysis." *Proteomics*, Vol. 5, Issue 9, 2412-2416.

Smith L, et al. (2006) "The analysis of doxorubicin resistance in human breast cancer cells using antibody microarrays." *Molecular Cancer Therapeutics*, Vol. 5, Issue 8, 2115-20.

# Expression Profiling

## Antibody Arrays

	<b>XPRESS Profiler725</b>	<b>Panorama® Antibody Microarray – Cell Signaling</b>	<b>Panorama Antibody Microarray MAPK and PKC Pathways</b>
<b>Catalog Number</b>	<b>XP725</b>	<b>CSAA1</b>	<b>MPAA3</b>
<b>No. of Elements</b>	725	224	84
<b>No. of Arrays/Kits</b>	2	2	2
<b>Application</b>	Protein expression profiling	Protein expression profiling	Protein expression profiling
<b>Product Descriptions</b>	The Panorama Antibody Microarray – XPRESS Profiler725 Kit is designed for studying protein expression in cell or tissue extracts. The antibody array contains 725 different antibodies each spotted in duplicate on nitrocellulose-coated glass slides. These antibodies represent families of proteins known to be involved in a variety of important biological pathways, including cell signaling, gene regulation, and MAPK/PKC.	The Panorama Antibody Microarray – Cell Signaling is designed for studying the relative level of protein expression in cell or tissue extracts. The array contains 224 different antibodies, each spotted in duplicate spots on nitrocellulose-coated glass slides.	Panorama Antibody Microarray – MAPK & PKC Pathways Kit is designed for studying protein expression in cell or tissue extracts. The antibody array contains 84 different antibodies, each spotted in duplicate on a nitrocellulose-coated slide.
<b>Content</b>	<ul style="list-style-type: none"> <li>■ Apoptosis</li> <li>■ Cell cycle</li> <li>■ Neurobiology</li> <li>■ Cytoskeleton</li> <li>■ Signal Transduction</li> <li>■ Nuclear Proteins</li> <li>■ Kinases in MAPK and PKC pathways</li> <li>■ Phosphatases in MAPK and PKC pathways</li> <li>■ Transcription factors</li> <li>■ Histone modifying enzymes</li> <li>■ Modified Histones</li> <li>■ Proteins involved in p53 pathways</li> <li>■ Mouse and Rat</li> <li>■ Total, Nuclear, and Cytoplasmic extracts, Brain, Lung, Heart, Skeletal Muscle, Stomach, Spleen, Testis, Thymus, Liver</li> </ul>	<ul style="list-style-type: none"> <li>■ Apoptosis</li> <li>■ Cell cycle</li> <li>■ Neurobiology</li> <li>■ Cytoskeleton</li> <li>■ Signal Transduction</li> <li>■ Nuclear Proteins</li> </ul>	<ul style="list-style-type: none"> <li>■ Kinases in MAPK and PKC pathways</li> <li>■ Phosphatases in MAPK and PKC pathways</li> </ul>
<b>Sample</b>	<ul style="list-style-type: none"> <li>■ Suspension culture: 10 million cells</li> <li>■ Adherent culture: 3 × 10 cm petri dishes</li> <li>■ Tissue: 500 mg</li> </ul>	<ul style="list-style-type: none"> <li>■ Suspension culture: 10 million cells</li> <li>■ Adherent culture: 3 × 10 cm petri dishes</li> <li>■ Tissue: 500 mg</li> </ul>	<ul style="list-style-type: none"> <li>■ Suspension culture: 10 million cells</li> <li>■ Adherent culture: 3 × 10 cm petri dishes</li> <li>■ Tissue: 500 mg</li> </ul>
<b>Detection Chemistry</b>	Cy™3/Cy5 or similar	Cy3/Cy5 or similar	Cy3/Cy5 or similar

## Antibody Arrays

Panorama Antibody Microarray – Gene Regulation I Kit	Panorama Antibody Microarray – p53 Pathways	Panorama Mouse/Rat Tissue Extract Protein Array Kit
<b>GRAA2</b>	<b>PPAA4</b>	<b>MRPA1</b>
112	112	62
2	2	3
Protein expression profiling	Protein expression profiling	Tissue expression profiling antibody reaction screenings
Panorama Antibody Microarray – Gene Regulation I Kit is designed for studying protein expression in cell or tissue extracts. The antibody array contains 112 different antibodies, each spotted in duplicate on a nitrocellulose-coated slide.	Panorama Antibody Microarray – p53 Pathways Kit is designed for studying protein expression in cell or tissue extracts. The array contains 112 different antibodies, each spotted in duplicate on a nitrocellulose-coated slide.	The Panorama Mouse/Rat Tissue Extract Protein Array Kit is designed for studying protein expression in different tissue extracts. Each spotted extract is pre-tested for its ability to bind antibodies in the array using several different antibodies. Each extract type is spotted at equal protein concentration depending on its origin i.e., total, cytoplasmic, or nuclear extract. All extracts were prepared in denaturing and reducing buffer and boiled.
<ul style="list-style-type: none"> <li>■ Transcription factors</li> <li>■ Histone modifying enzymes</li> <li>■ Modified Histones</li> </ul>	<ul style="list-style-type: none"> <li>■ Proteins involved in p53 pathways</li> </ul>	<ul style="list-style-type: none"> <li>■ Mouse and Rat</li> <li>■ Total, Nuclear, and Cytoplasmic extracts; Brain, Lung, Heart, Skeletal Muscle, Stomach, Spleen, Testis, Thymus, Liver</li> </ul>
<ul style="list-style-type: none"> <li>■ Suspension culture: 10 million cells</li> <li>■ Adherent culture: 3 × 10 cm petri dishes</li> <li>■ Tissue: 500 mg</li> </ul>	<ul style="list-style-type: none"> <li>■ Suspension culture: 10 million cells</li> <li>■ Adherent culture: 3 × 10 cm petri dishes</li> <li>■ Tissue: 500 mg</li> </ul>	<ul style="list-style-type: none"> <li>■ Serum, 1:200 dilution</li> <li>■ Hybridoma supernatant, 1:2 dilution</li> <li>■ Ascites fluid, 1:100-1:1,000 dilution</li> <li>■ Purified antibody, 10-20 µg/ml</li> <li>■ Fractionated serum, 1:200-1:1,000 dilution</li> </ul>
Cy3/Cy5 or similar	Cy3/Cy5 or similar	Cy3/Cy5 or similar