

# Panorama™ Ab Microarray Cell Signaling Kit

*The Next Step in Expression Analysis*

Easy and Fast  
Protocol

Convenient Cy3/Cy5  
Microarray Format

Superb Spot  
Morphology for  
Reliable Data Analysis

No Expensive or  
Unusual Equipment

[sigma-aldrich.com](http://sigma-aldrich.com)



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# Protein Profiling Made Easy

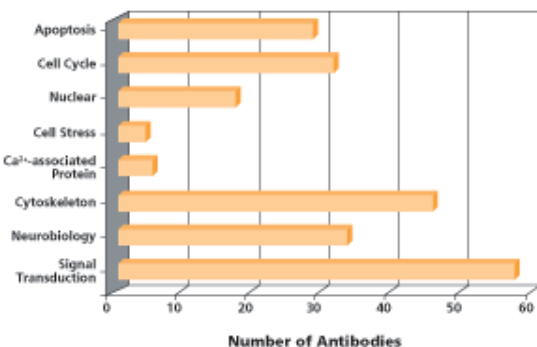
Whether you are an experienced DNA microarray user and are ready to take the next step or are new to microarrays and eager to jump right into protein profiling, the **Panorama™ Ab Microarray – Cell Signaling Kit** is the right tool for you. Containing 224 antibodies to key cellular proteins (with a special emphasis on cell signaling proteins), this array can provide answers to cell biology questions not obtainable from any DNA microarray.

## The Antibodies

- Are each extensively validated for use on the array
- Are arrayed on nitrocellulose-coated glass slides
- React with samples from human, mouse and rat
- Include several that are phosphospecific for their target proteins
- Are also available separately from Sigma-Aldrich
- Are listed by functional class and can be found at [sigma-aldrich.com/antibodyarray](http://sigma-aldrich.com/antibodyarray)

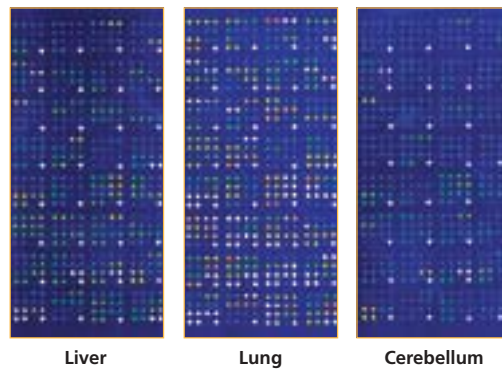
## Protein Functional Classes

While the greatest number of proteins represented in the array are from the Signal Transduction functional class, there are 7 other important functional classes also represented.



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

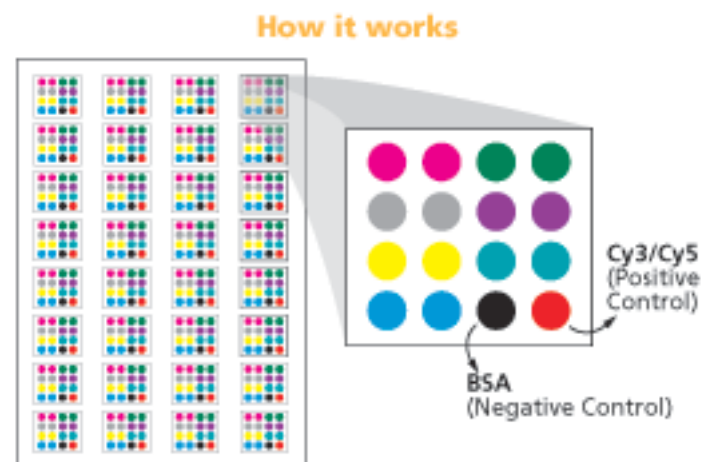
## Expression Differences Within Mouse Tissues



Using the **Panorama Ab Microarray – Cell Signaling Kit**, proteins are observed to be differentially expressed in mouse tissues. Protein extracts were prepared from mouse liver, lung, and cerebellum tissue samples using Extraction/Labeling Buffer. One mg of each protein extract was labeled with Cy3 and incubated on separate arrays at a concentration of 5 µg/ml, as described in the kit protocol.

## Array Organization

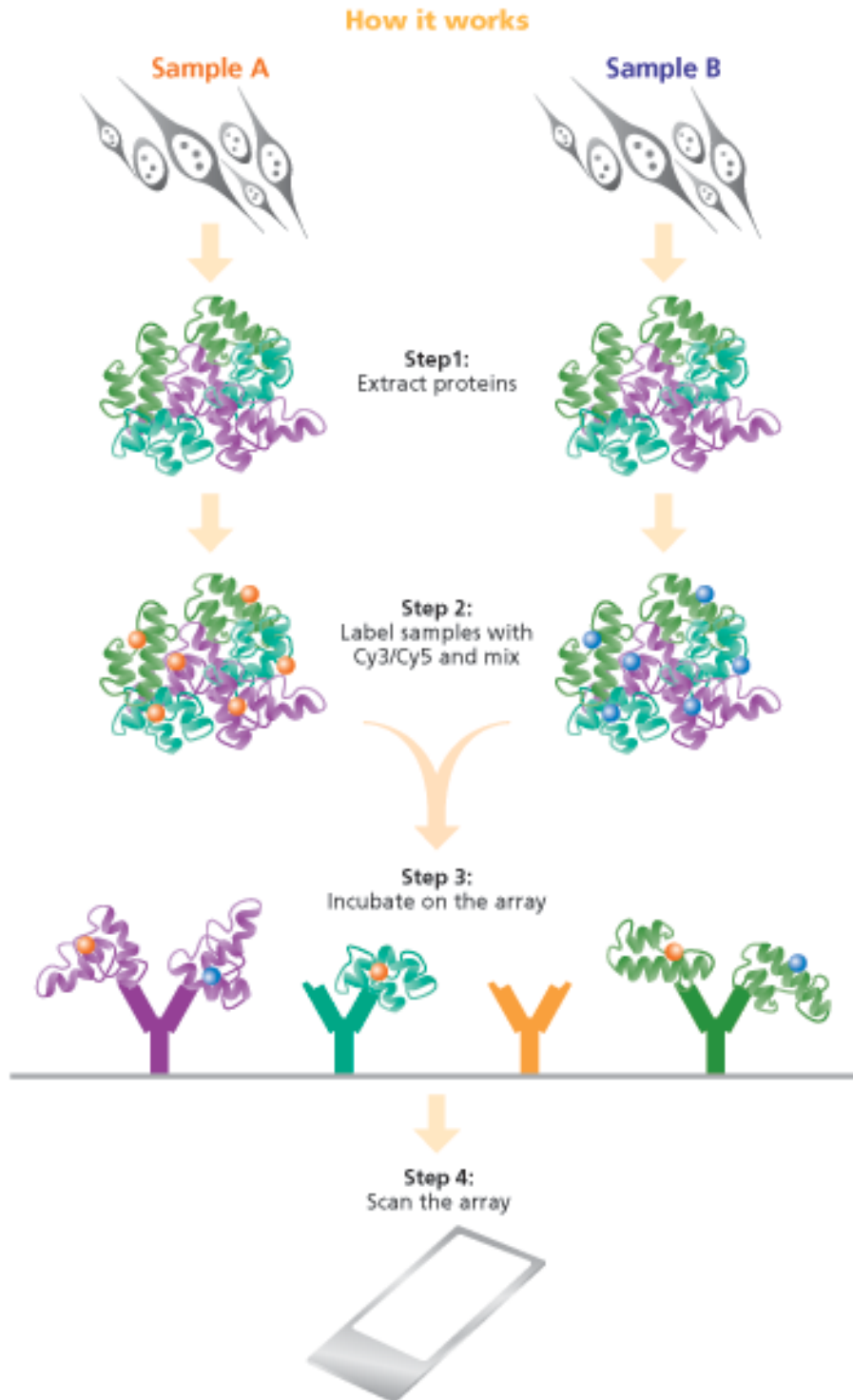
To facilitate reliable interpretation of array experiments, each antibody feature is double-spotted and each of the 32 fields contains its own positive and negative control.



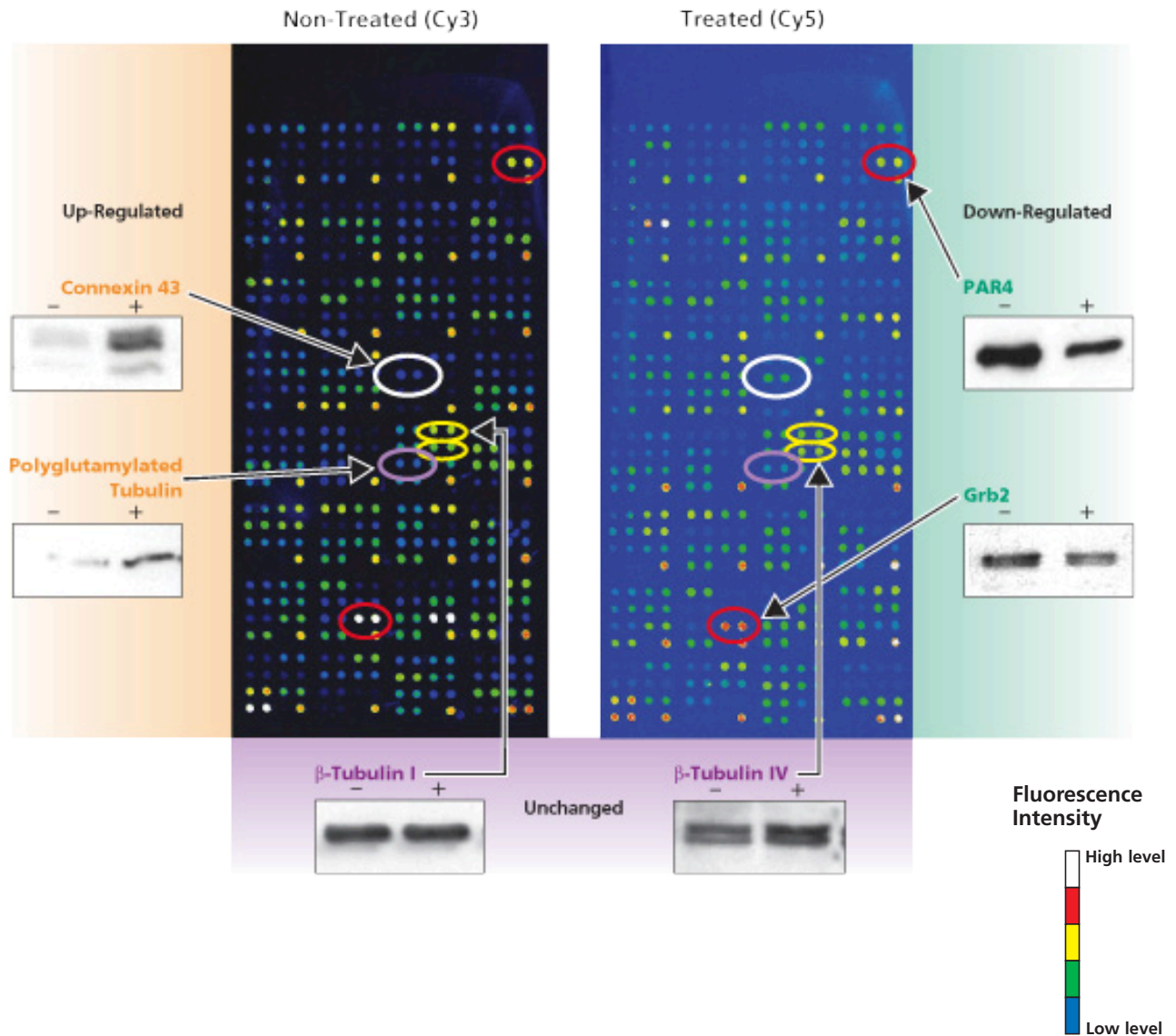
## How It Works

Following extraction of proteins from cells or soft tissues, the protocol is similar to a DNA microarray experiment and requires the same equipment. The two protein extracts are labeled separately with either Cy3 or Cy5. After the removal of excess label, the labeled pro-

tein 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.



## Induction with Retinoic Acid in Mouse F9 Cells



Using the **Panorama Ab 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 Cy3 and Cy5, respectively. A mixture containing equal amounts of each labeled extract ( $5 \mu\text{g/ml}$ ) 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.

(Note that the blue background for Cy5 represents the normal and unavoidable interference of the nitrocellulose membrane.) Changes in expression level were confirmed by immunoblot, as indicated. See details below.

Equal amounts of protein extract ( $20 \mu\text{g}$  per 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.

## Panorama™ Ab Microarray – Cell Signaling Kit



### Ordering Information

Product Code	Product Description
CSAA1	Panorama Ab Microarray — Cell Signaling 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



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