# Multiplex protein expression profiling with Panorama™ antibody arrays

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## Introduction

Antibody microarray technology has become a central tool for the identification, quantitation and functional analysis of proteins. This high throughput proteomic technology enables efficient and sensitive protein analysis thus accelerating protein profiling studies for biomarker discovery (Kopf, E. and Zharhary, D., 2007).

## Material and methods

Panorama™ Antibody Arrays are kits that contain nitrocellulose-coated glass slides spotted with 84 to 725 antibodies. The series includes arrays specific for Cell Signaling proteins (Product code CSAA1), for MAPK & PKC Pathway proteins (Product code MPAA3), and for Gene Regulation proteins important in chromatin remodeling (Product code GRAA2). There is also an array for p53 pathways proteins (Product code PPAA4), as well as a large array with 725 antibodies representing many biological pathways (Product code XP725). Protein extract samples from human, mouse, or rat cells and tissues can be assayed using these arrays. The extracts are labeled with Cy3 or Cy5 fluorescent dyes and then applied to the array. After a short incubation and wash, the array is scanned and numerical values of the fluorescence intensity are obtained. Numerical values for each spot are normalized against that of antibodies to house-keeping gene proteins present on the array, to eliminate the effect of dye conjugation efficiency. Differences in protein expression between two samples can be assayed on one array by labeling samples with different dyes (Cy3 or Cy5) and applying a mixture of both on the array.

## Results

Many applications of the Panorama™ Ab Microarray family of products have been reported. They were employed in identifying proteins involved in differentiation of F9 embryonic mouse stem cells by retinoic acid (Kopf, E., et al., 2005), and in the identification of proteins involved in extracellular and intracellular signaling components of the mammary adipose tissue and its interstitial fluid in high-risk breast cancer patients (Celis, J.E., et al., 2005). Other applications were the identification of proteins involved in drug resistance (doxorubicin) in human breast cancer cells (Smith, L., et al., 2006), and the identification of the cellular pathways involved in the maintenance of human embryonic stem cell pluripotency and viability (Armstrong, L., et al., 2006). Antibody arrays are also useful in studying the effect of gene silencing by siRNA as demonstrated using LAP2β gene silencing as a model system. The antibody array was also used in protein/protein interaction studies to identify β-catenin binding proteins in NIH-3T3 cells. All array results were confirmed by using other immunochemical assays such as immunoblotting, immunoprecipitation or immunocytochemistry.

## References

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