

proteomics

Panorama™ Human p53 Functional Protein Array: A Useful Tool for Accurate Identification of p53 Interactions

By Henry Hepburne-Scott,¹ Klaus Herick,² and Jodi Zobrist³

¹Procognia Ltd, Maidenhead, UK

²Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany

³Sigma-Aldrich Corporation, St. Louis, MO, USA

- Full protein functionality – proprietary tagging technology ensures only functional p53 proteins are immobilized
- Accurate representation of 49 p53 SNP variants
- Compatible with wide range of probes – DNA, proteins, kinases, or small molecules
- Suitable with CyTM3/Cy5-based detection chemistries

Introduction

The completion of the Human Genome Project in 2003 has brought about the great challenge of identifying functions for the estimated 30,000 proteins that are encoded in the human genome. When considering various splice variants and post-translational modifications, there is an expected 1,000,000 proteins to consider for this complex biological system. By enabling parallel analysis of multiple samples, microarray platforms have remarkably accelerated protein identification and characterization.

Using functional protein microarrays eliminates the process of expressing a protein followed by immobilization on a solid support. Researchers are now able to focus on elucidating interactions that occur between the target protein and various probes such as small molecules, DNA, or other proteins. The Panorama™ Human p53 Protein Functional Microarray (Product Code HPFM1) is designed to establish protein interactions against a target probe in as little as 3 hours (Figure 1).

The Panorama Human p53 Protein Functional Microarray, which contains 49 SNP variants and one wild-type control, utilizes a proprietary tagging technology based on the presence and biotinylation of a biotin-carboxy carrier protein (BCCP) tag to ensure only correctly folded and fully functional proteins are immobilized. The tagging system utilizes a 50 Å spacer arm, thereby, allowing proteins to be presented in a similar manner and maximizing the opportunity for active sites to interact with binding partners (Figure 2).

DNA Binding Assay

Double-stranded Cy3-labeled GADD45 promoter DNA (upper strand 5'-GTACAGAACATGCTAAGCATGCTGGGGAC-3') was screened against the p53 array to determine protein functionality. GADD45 DNA is known to bind wild-type p53 and to various extents will bind p53 mutants. Following the protocol described in the technical bulletin, the intensity of fluorescent signals for mutant p53 was compared to wild-type p53. Slides were scanned using the PerkinElmer® ScanArray® Express instrument. Quantitation was performed using ScanArray Express software and data obtained was normalized to binding exhibited by wild-type p53 (Figure 3).

Antibody Binding Assay

Cy5-labeled monoclonal p53 antibody (Product Code C6594) was applied to the array as a positive control to confirm mutant and wild-type p53 protein was immobilized. Slides were scanned using the PerkinElmer ScanArray Express instrument. Quantitation was performed using ScanArray Express software and data obtained was normalized to binding exhibited by wild-type p53 (Figure 4).

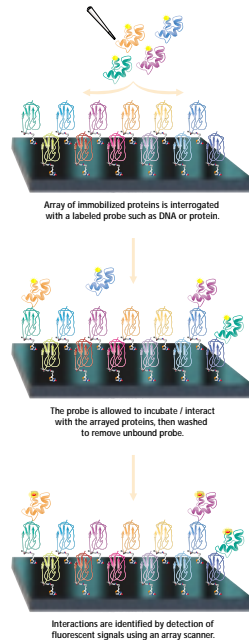


Figure 1. Overview of p53 array workflow.

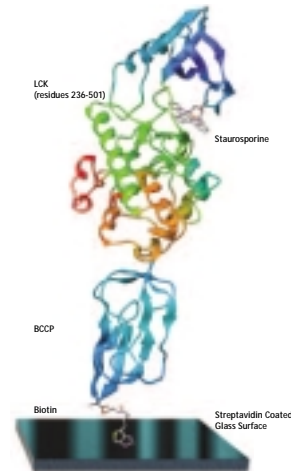


Figure 2. Schematic of biotin-BCCP-protein. The BCCP tagging technology ensures only correctly folded and fully functional proteins are immobilized. The BCCP-tagged Lymphocyte-Specific Kinase (LCK) is demonstrated. The presented LCK represents residues 236-501 out of 509 total residues. The LCK domain is complexing Staurosporine, a broad specificity kinase inhibitor.

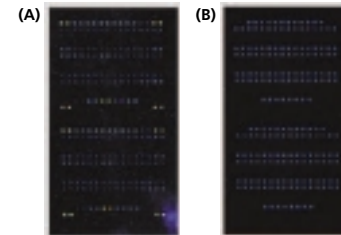


Figure 3. Array image for DNA binding, followed by anti-p53 binding on the same array. (A) Some of the mutations of p53 affect the core-binding domain for DNA as demonstrated with a GADD45-Cy3 DNA probe. (B) Probing with an anti-p53-Cy5 antibody indicates that every spot contains p53 protein.

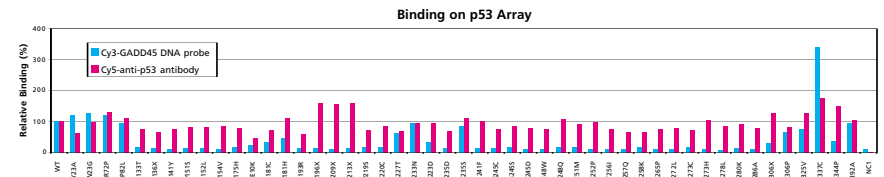


Figure 4. Relative DNA probe binding and p53 conformation independent antibody binding to p53 mutants. Data (RFU) from Figure 3 were normalized to wild-type p53. Some mutations of p53 affected the binding of GADD45-Cy3 (blue bar), while others demonstrated little or no change in DNA binding activity (e.g. P82L). Both decreased (e.g. M113T) and increased (e.g. R337C) DNA binding was observed. Subsequent to the DNA binding assay, a Cy5-anti-p53 antibody (pink bar) was incubated on the array to confirm the relative amount of each spotted p53 protein.

Summary

Functional protein microarrays are emerging as a powerful tool for the investigation of protein populations and protein interactions. The Panorama Human p53 Functional Microarray invokes the advantages of the BCCP tag to ensure the proteins spotted are functional and optimally displayed for binding interactions. The Panorama format overcomes traditional limitations of independent analysis (i.e. time and accessibility of immobilized protein) by allowing efficient parallel analysis of target probe against multiple proteins.

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Ordering Information

| Product | Description | Unit |
|---------|---|-------|
| HPFM1 | Panorama Human p53 Protein Function Microarray | 1 kit |
| HPFM2 | Panorama Human Cancer Array v1 Microarray | 1 kit |
| CSAA1 | Panorama Antibody Microarray – Cell Signaling | 1 kit |
| GRAA2 | Panorama Antibody Microarray – Gene Regulation | 1 kit |
| MPAA3 | Panorama Antibody Microarray – MAPK & PKC Pathways | 1 kit |
| MRPA1 | Panorama Mouse/Rat Tissue Extract Protein Array Kit | 1 kit |