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ProArray Ultra™

A new protein and peptide microarray with true analytical grade performance

Do you need to increase the throughput of your ELISA assays and do more with less sample at a lower cost? ProArray Ultra™ delivers a combined protein and peptide array platform with unrivalled scalability and flexibility, while exceeding the signal quality and performance of optimized ECL ELISA assays.

The Turnkey Solution to Your Epitope Discovery Program

Flexibility, scalability and sensitivity are the hallmarks of the ProArray Ultra™ platform. Adding real value to your research projects is the additional element brought to you by our customer service team of PhD immunologists and biochemists. Discuss your project with us and we will design an assay and array format tailored to achieve your objectives. The ProArray Ultra™ technology is the turnkey solution to your epitope mapping and discovery programs.

Key Features of the ProArray Ultra™ Platform

- More than 30,000 proteins or peptides per array
- Less than 1 µl sample required for a typical analysis
- Signal performance better than ECL ELISA and comparable to MSD®
- Flexibility to immobilize any protein or peptide
- 1-24 replicate sub-array formats for multiple applications
- Delivered as a rapid turnkey service with comprehensive report

ProArray Ultra™ has been specifically developed as a combined peptide and protein array platform; combining the epitope/interaction site resolving power of scanning peptide arrays with that of high throughput and protein interaction screening.

ProArray Ultra™ achieves full compatibility for overlapping peptide libraries and proteins, and uses a flexible peptide synthesis platform to enable printing of arrays optimized for specific applications. Moreover, our design improvements result in greater uniformity of spot morphology between peptides and proteins, reduced background signal and improved assay performance in terms of sensitivity, dynamic range, and variance.

The result is a protein array platform that is comparable in analytical performance to MSD® technology and that outperforms ECL ELISA assays, while at the same time tens of thousands of interactions can be detected in a single experiment. More importantly only microliter amounts of sample are required for each analysis, enabling projects using limited amounts of sample that are simply impossible to carry out with 96-well plate-based assays.

Applicable Program Areas

The ProArray Ultra™ technology is appropriate for a range of programs and projects, including:

- Antigen discovery
  - Reverse immunology on proteins or entire pathogen proteomes
  - Screening sera from disease state or convalescent donors against overlapping peptides
- Mapping of monoclonal antibody binding sites
  - Mapping is linear in the first instance (unless conformational peptides are used), but strong conformational epitopes will be detected in linear sequences too
  - The platform also allows for the use of constrained peptides, e.g. cyclized peptides
- Understanding the epitopes presented and recognized
  - Disease progression cycle
  - Pathogen life cycle and recovery phases
- Batch release testing: vaccines, biologics
- Evaluate post-translational modifications on binding (large scale potential)
  - Highly flexible regarding peptide synthesis and available modifications
- Enzyme interaction site mapping: kinases, phosphatases
- Biomarker discovery and tracking
  - Measure potential biomarkers and/or surrogate endpoint markers
  - Measure changes in patient samples over time
  - Measure multiple biomarkers - simultaneous assessment of a range of identified markers of interest
- Cytokine detection in samples
- Iterative optimization of peptides

Performance Data

The design of ProArray Ultra™ overcomes the compromise between analytical quality multi-well immunoassay platforms and the scalability of existing protein and peptide array technology. Evaluation of our new design features against other more conventional assay methodologies, such as optimized ECL ELISA and the Meso Scale Discovery (MSD®) technology shows that ProArray Ultra™ compares favourably to both plate-based platforms, and has lower background and better overall signal to noise performance (figure 1).

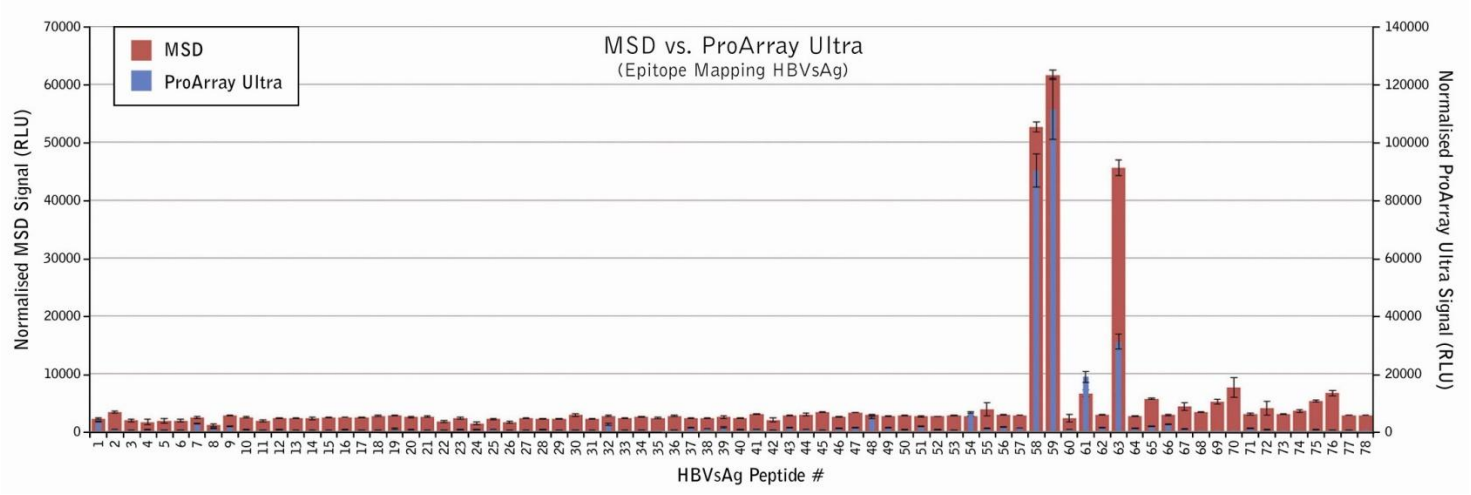

Figure 1: Results of a direct comparison of ProArray Ultra™ (blue bars) and high-performance 96-well MSD® assay technology (red bars) for identical peptide libraries derived from the well-characterized Hepatitis B Virus surface Antigen (HBVsAg) incubated with the same anti-HBVsAg seropositive serum sample. All data include error bars reflecting standard error of the mean (SEM) values. ProArray Ultra™ compares favourably to the MSD® plate-based platform, and has lower background and better overall signal to noise performance. The peptides identified as epitopes by ProArray Ultra™ correlate with results from the MSD® assay.

The MSD® technology platform is suitable for evaluation of relatively small numbers of analytes with a large numbers of samples. In contrast, ProArray Ultra™ can accommodate a large number of peptide or protein analytes (>30,000) on a single slide, for analysis with sample volumes as low as 1 µl for sub-grid formats.

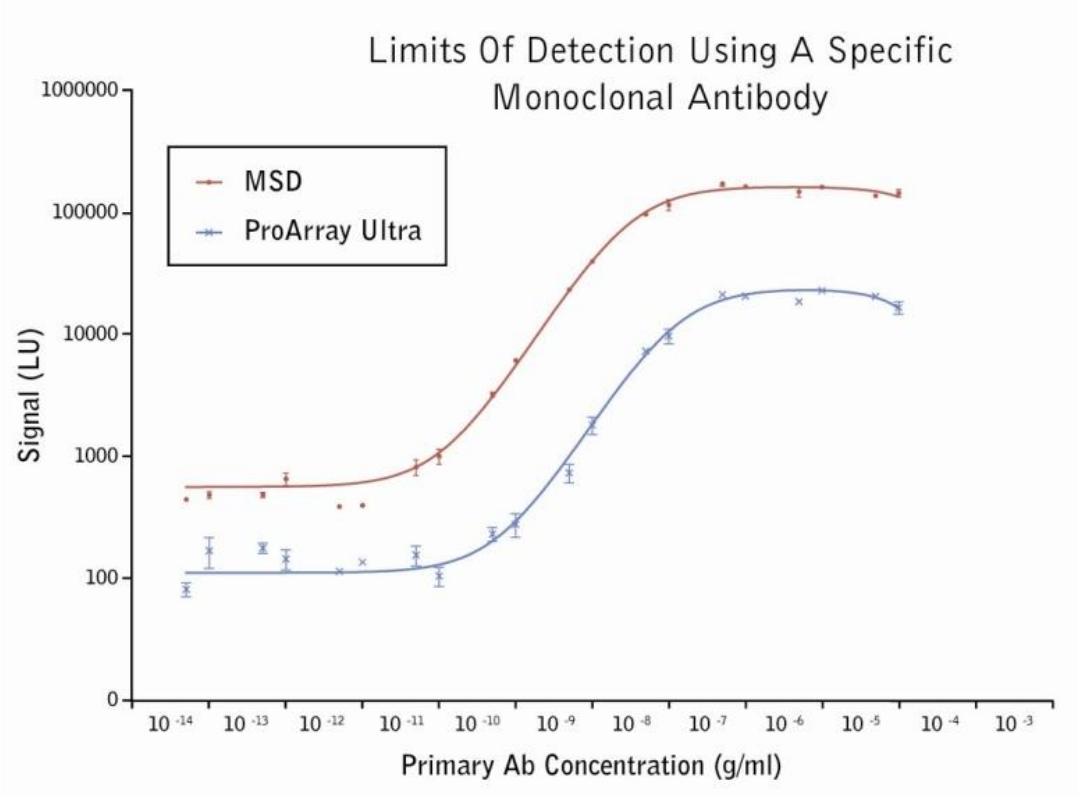

Figure 2: Limit of Detection (LOD) comparison for MSD and ProArray Ultra™ using a specific monoclonal antibody. A saturation curve profile was obtained with a linear range of approximately 2.5 logs for both ProArray Ultra™ and MSD® assay. Very low signal variation was found for both technologies in terms of signal detected. The limit of physical detection can be seen as ~0.5ng/ml.

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Flexible Array Design

Our printing technology confers complete flexibility for design around your requirements.

The ProArray Ultra™ microarray setup is based on five printing layouts of 1, 2, 4, 8 or 24 identical sub-arrays per slide. The slides can be overlaid with a separation gasket allowing each sub-array to be incubated with an individual antibody-sample, i.e. up to 24 separate samples can be analyzed per slide.

The exact print setup is based on the number of peptides or proteins being probed, the number of sample repeats required, and the number of technical repeats required (at least 3 are suggested, up to 6 are recommended). All these factors are taken into account to create the optimal printing layout.

The five different print setups can accommodate a large range of custom printed features (peptides & proteins) alongside standard control spots.

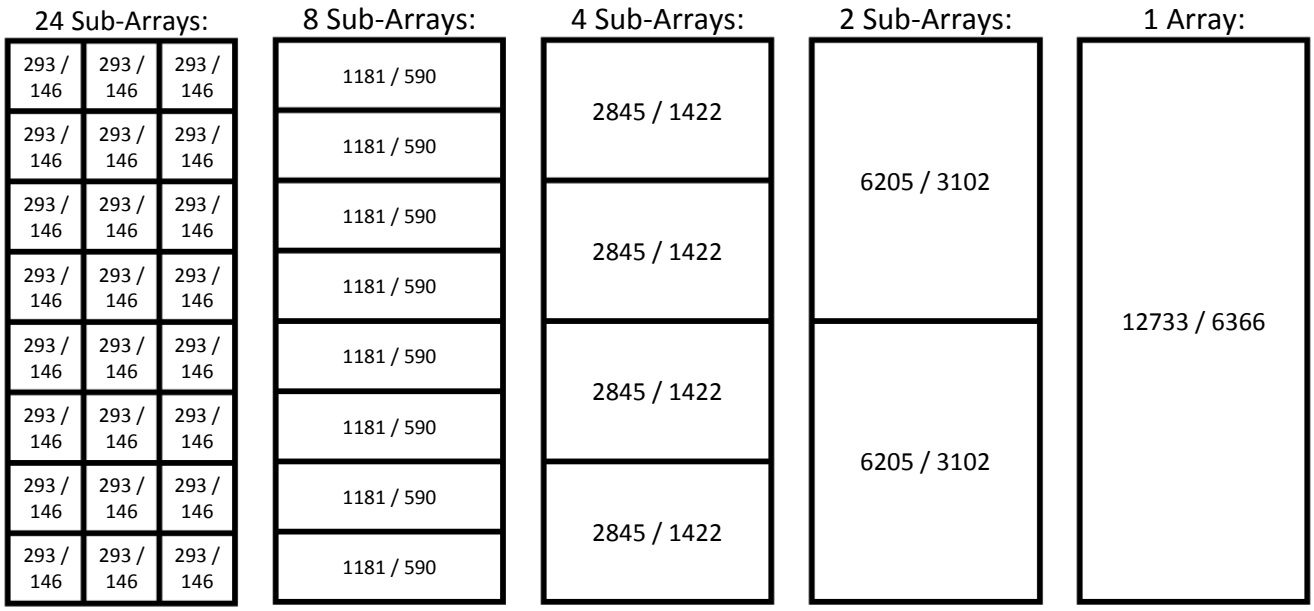

Figure 3: The diagram shows 5 possible layouts of the microarray printing design. The first number in each pair indicates the number of samples that can be spotted in triplicate; the second number indicates the number of spots in sextuplicate. The sub-arrays can be physically separated from each other using specially fitted gaskets, allowing incubation of each discrete sub-array with a different sample.

Consistent High Performance for Peptides and Proteins

ProArray Ultra™ has been developed to minimize spotting variation in terms of spot size and morphology, and further optimized to ensure comparability between peptides and proteins on the same slide (a major concern unsatisfactorily addressed by first generation peptide microarrays). Figure 4 emphasizes the level of consistency achieved across a range of titrations for the ligand protein.

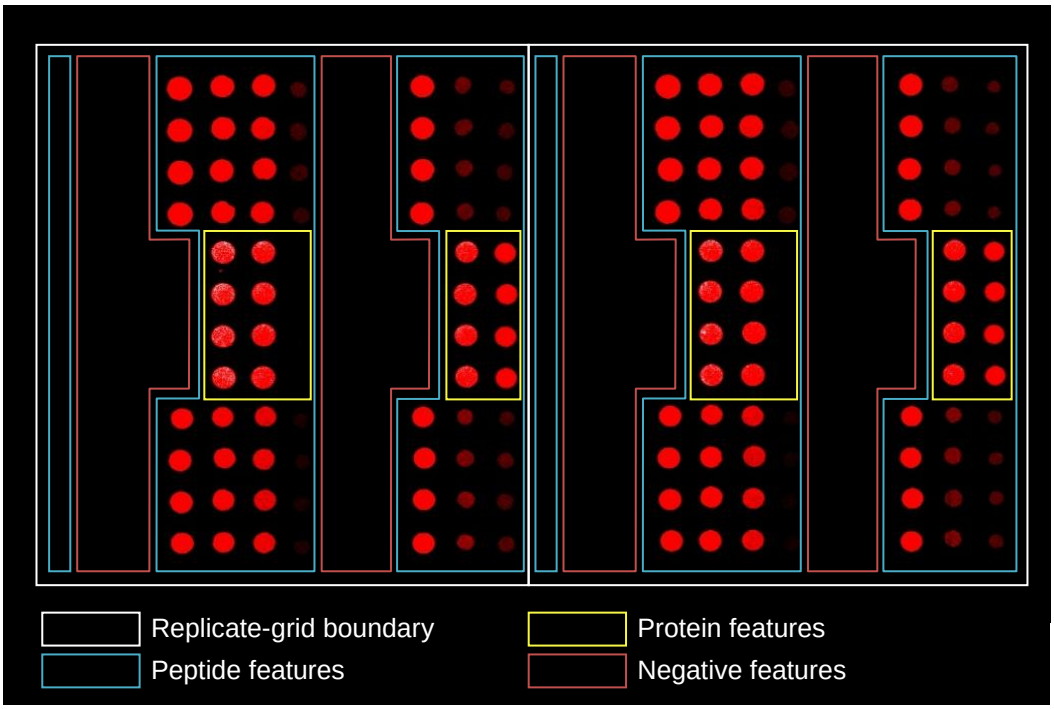

Figure 4: ProArray Ultra™ peptide microarray imaged at 5µm resolution. Peptides and protein samples are titrated and spotted in quadruplicate rows, in duplicate sub-grids, and detected with purified antibody. The array photograph shows peptide-antibody interactions with signal intensities from high to low, reflected by gradations in colour from red (high signal) to black/dark red (low signal). The change in signal intensity (high to low) reflects a decrease in the amount of analyte spotted onto the array. The image confirms that both peptide and protein spots are of a consistent high quality, reinforcing the suitability of the platform for multiple applications.

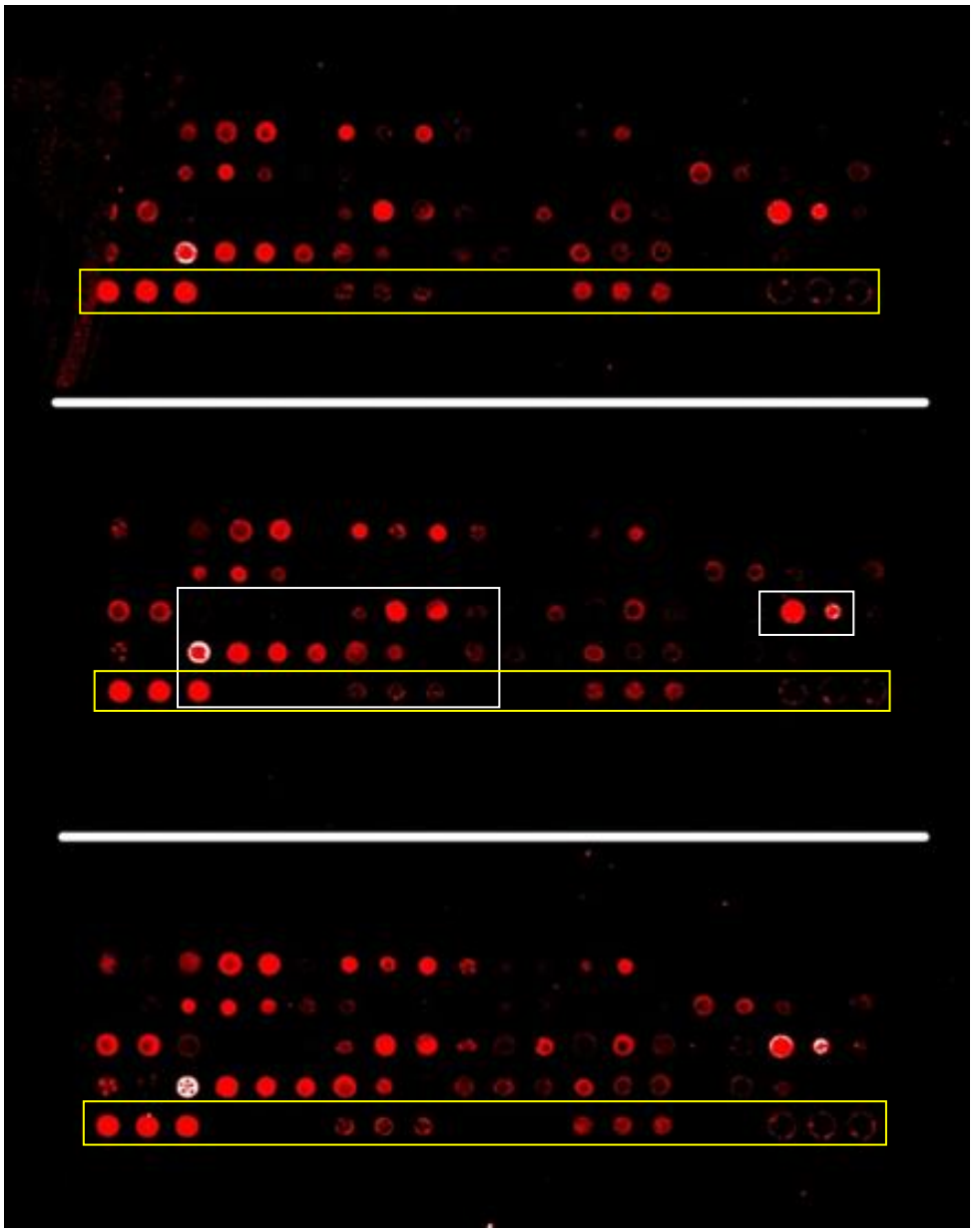

Figure 5: Peptide microarray image from a leading alternative “A”.
Alternative “A” peptide microarray imaged at 5µm resolution. Triplicate grids are shown together for clarity and cross-grid comparison purposes. These image data reflect 78 overlapping peptides derived from a single immunogenic protein printed with triplicate control protein features (yellow box) in triplicate grids. Significant variations are clearly visible in terms of spot size and morphology (white boxes).

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Technology Comparison

	ProArray Ultra™	MSD®	ECL ELISA
Considerations and Capabilities	Very high throughput ~ 1µl serum typically > 30,000 features possible Up to 24 serum samples per slide or a full dilution range of 1 or more serum samples. Modified/constrained/ long sequences possible	0.5 µl - 2.5 µl serum for up to 10 unique features per well Low throughput (~10 epitopes per plate) Limited epitope characterization; fixed configuration, limited to pre-determined test markers Good for multiple serum samples	5 µl serum volume per feature analyzed Low throughput (~ 30 epitopes per plate in triplicate)
Sensitivity *	0.5 ng/ml	0.05-10s of ng/ml	100s of ng/ml
CV (%) at high signal level	< 10%	5-10 %	~ 20%
Dynamic range*	2.5 logs	3 - 4.5 logs 1-10^6 maximum range	1-2 logs
Binding or Spotting of Peptides and Proteins	Compatible for proteins and peptides Peptides' individual physico-chemical properties are adaptively maintained by the protein conjugation approach	Proteins and peptides System is not specifically designed for immobilization of peptides	Proteins and peptides Can be adapted to accept any peptide

*Detection of antibodies (0.5 – 1 ng/ml) provides clinical relevance

ProArray Ultra™: A flexible multi-parameter microarray for the sensitive detection and characterization of peptide-protein and protein-protein interactions.

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