

Nucleic Acid and Protein Arrays on NUNC™ ArrayCote™ 16 Well Slides and 96 Well Plates

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Abstract

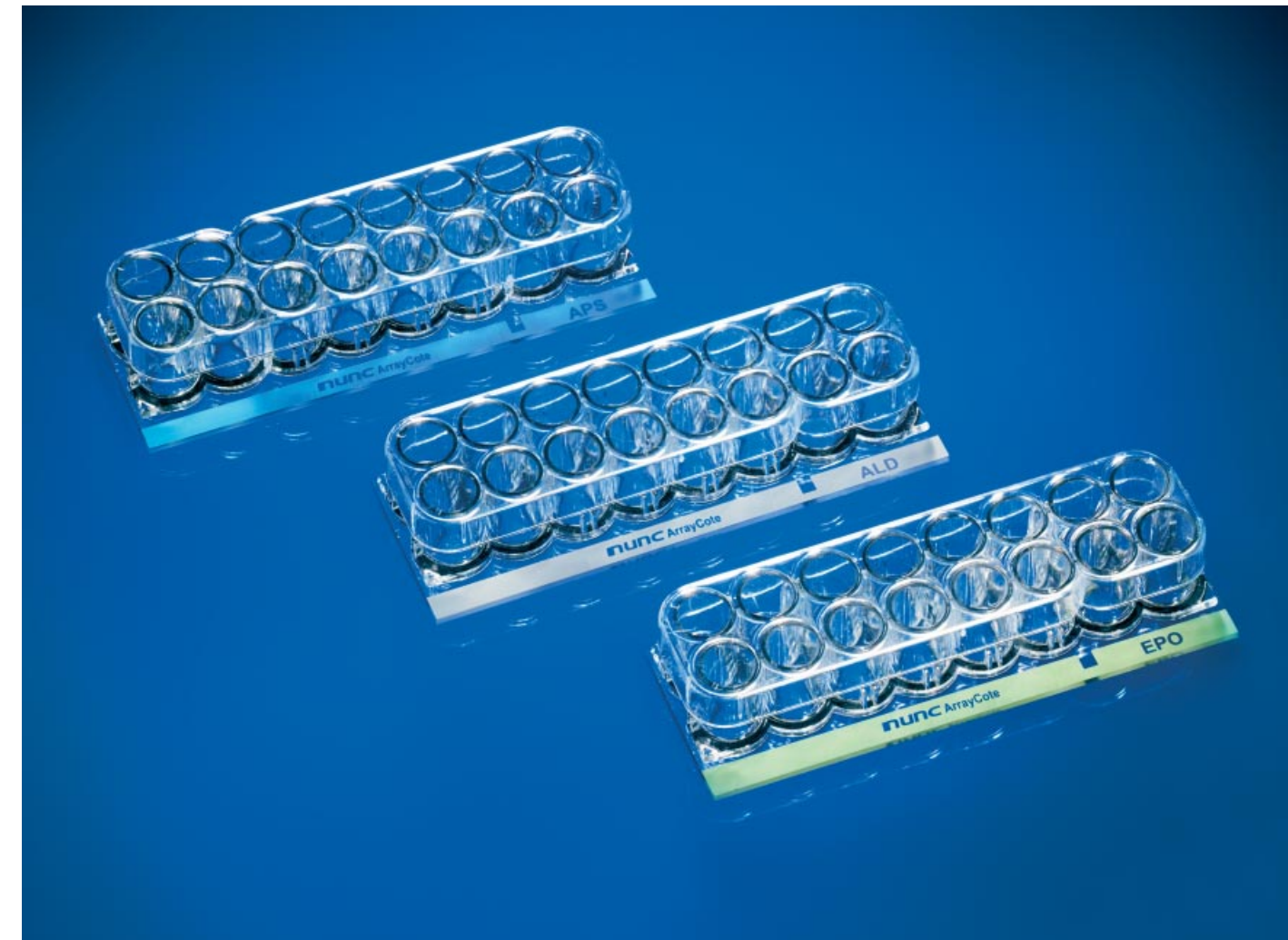
Microarrays offer an extremely powerful technique for sample analysis, but sample replication is often limited. Smaller, focused arrays provide a significant cost advantage, allowing a trade between the expense of high density and the statistical power from multiple replicates. By combining the replicate sampling possible in a chambered format with chemistries familiar to microarray users, NUNC has extended array technology in the form of 96 well plate and 16 well slide array platforms with excellent performance characteristics. With this format it is possible to spot either nucleic acids or proteins with excellent spot morphology (protein spots less than 250µm, PCR product spots less than 120µm) and good binding capacity (saturated signals at less than 0.2µg/ml probe concentration). Background levels are low, yielding substantial signal/noise ratios.

Introduction:

In spite of progress in genomics and proteomics, the pace of discovery has been hampered by an inability to assess a global picture of molecular conditions that drive cell and organism biology. Microarrays allow researchers to measure the level of transcription from all of an organism's genes simultaneously. Multi-condition gene arrays accelerate the identification of the proteins key to biological processes and diseases. Once this list (typically hundreds or tens of transcripts) is established, more traditional methods of cell and molecular biology that will include replicate sampling and testing must be used to create further knowledge.

Subsequent to establishing a profile of genes regulated under the conditions of interest, mega-arrays of thousands of sequences can be cost- and time-efficient, especially when hundreds or thousands of replicate samples must be taken to achieve statistical power. Smaller arrays, which cover the complete set of regulated, relevant transcripts, are more useful and more cost efficient. In addition, the use of array technology for diagnostic applications requires a smaller, more cost-efficient format. For this reason, NUNC has developed a series of formats designed for smaller arrays called the ArrayCote™ series.

ArrayCote glass slides and plates are designed with upper wells for intensive genomic and proteomic analysis. Chambered array products permit analysis of multiple samples or conditions on the same slide or plate. Aminosilane, aldehyde and epoxy surfaces are optimized for a variety of leading array applications.



16 Well ArrayCote Slides

- Aminopropylsilane, Epoxy, and Aldehyde surfaces
- Lidded, removable 16 well chamber on standard 1" x 3" glass slide
- Compatible with standard microarray scanners

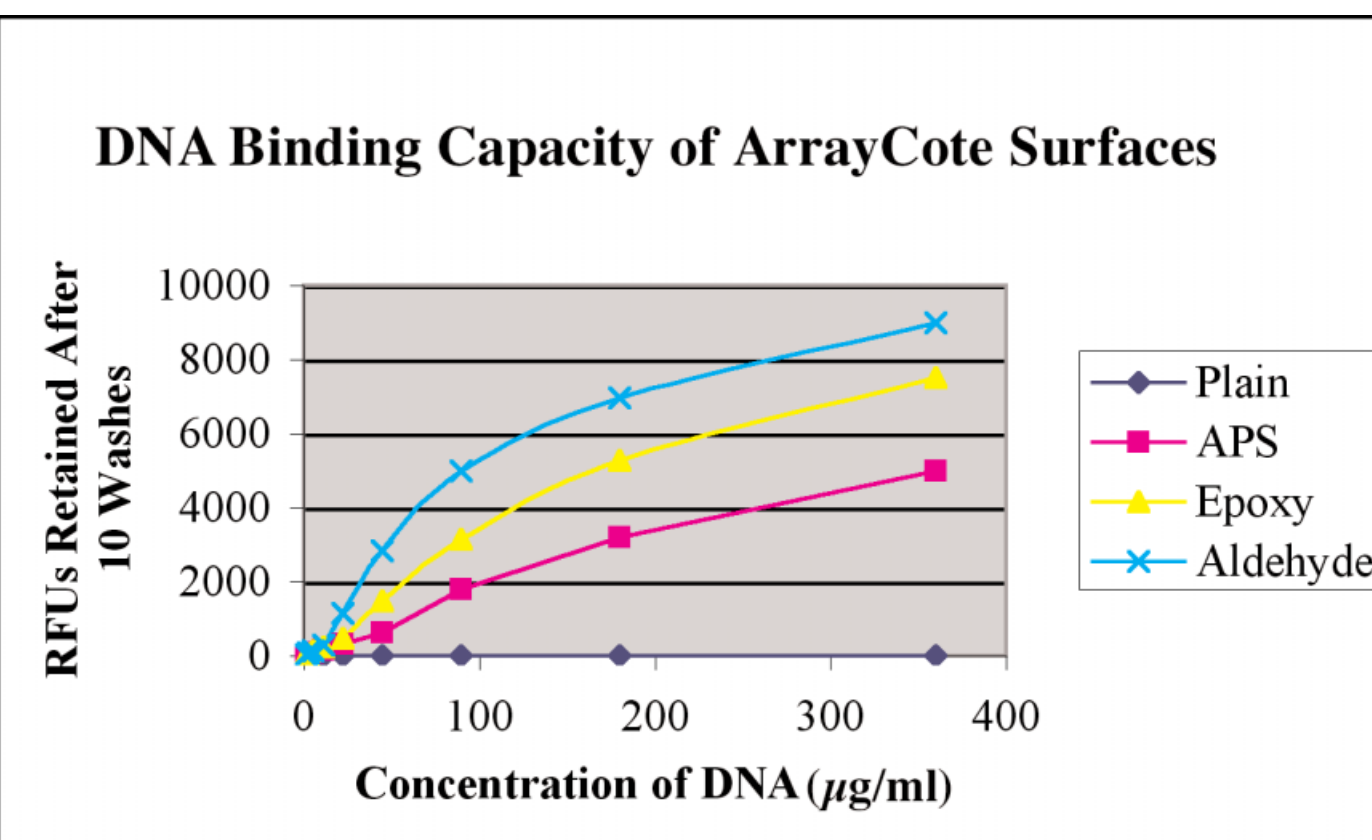


Figure 1. Enhanced DNA Binding Capacity on ArrayCote Surfaces

Spotting: 1µl drops of Cy3 labeled 20bp oligonucleotides in 50% DMSO/dH₂O (concentrations as noted in the figure) were spotted onto the center of wells of 96 well ArrayCote and plain glass plates. Spots were allowed to dry for 24 hours in a vacuum desiccator, fixed for 2 minutes at 302nm., and washed 10X in dH₂O. Fluorescence intensity was measured with an excitation filter of 550nm and emission filter of 570nm using a Victor Wallac fluorescence plate reader.



96 Well ArrayCote Plates

- Aminopropylsilane, Epoxy, and Aldehyde surfaces
- 96 well upper structure with coverglass bottom
- Compatible with standard microplate array scanners

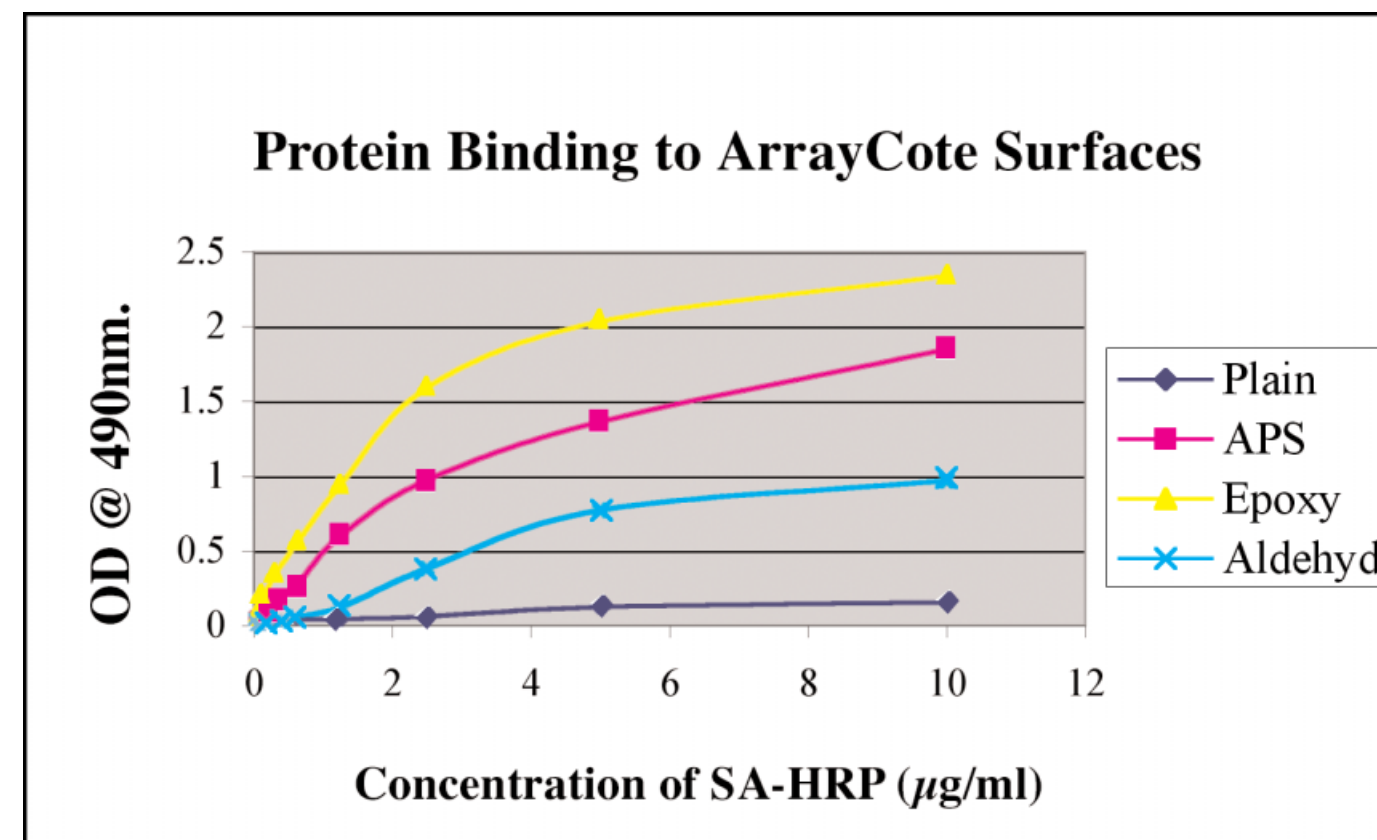


Figure 3. Enhanced Protein Binding Capacity on ArrayCote Surfaces

Binding: Serial dilutions from a 10µg/ml solution of streptavidin-HRP conjugate (SAHRP) (Pierce) were prepared in PBS + 0.1% Tween-20. 50µl were dispensed by columns into 96 well Epoxy, Aldehyde, and plain 96-well plates. The solutions were incubated in contact with the glass for one hour and then washed 5X with PBS + 0.1% Tween-20. Plates were developed with OPD.

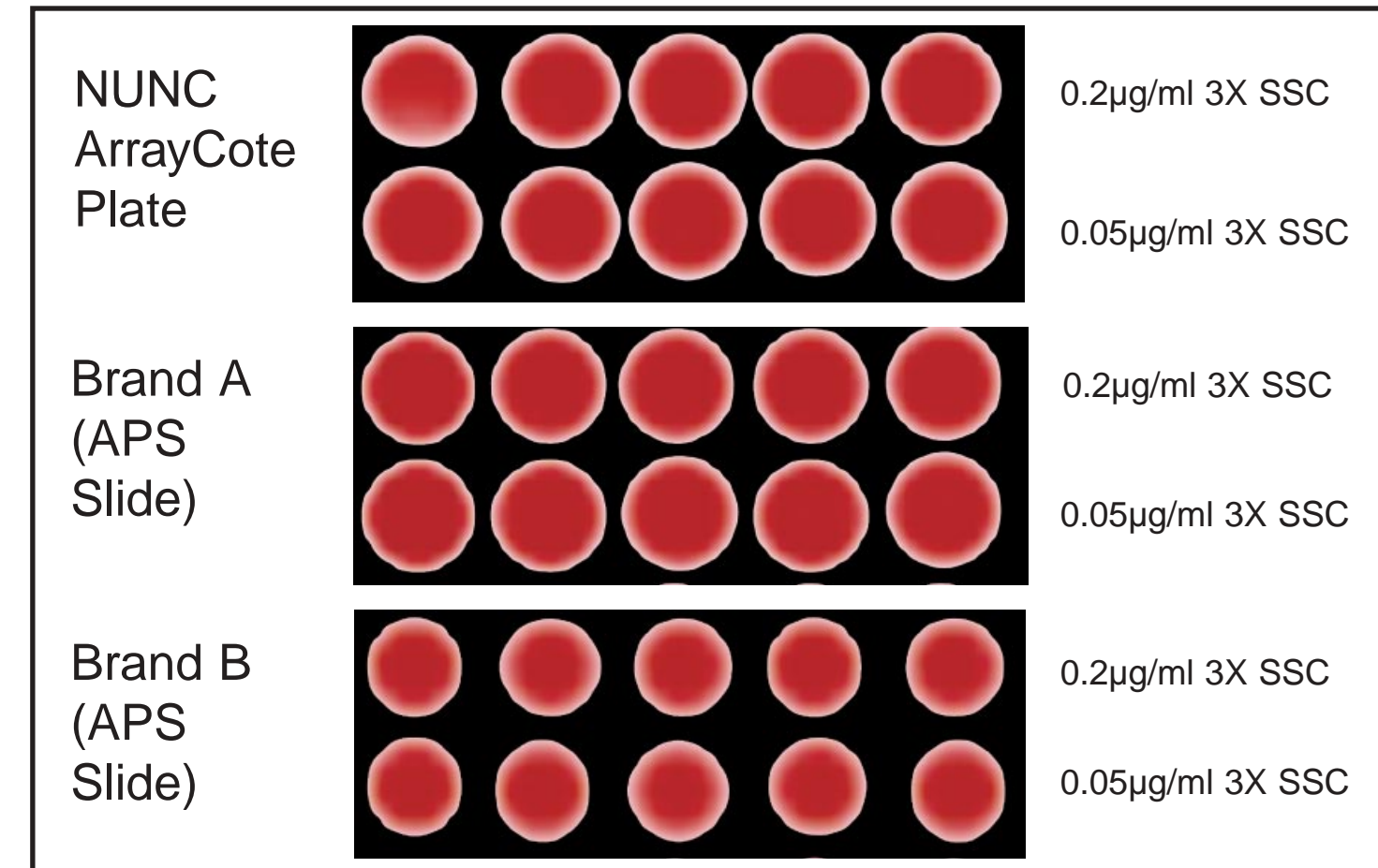


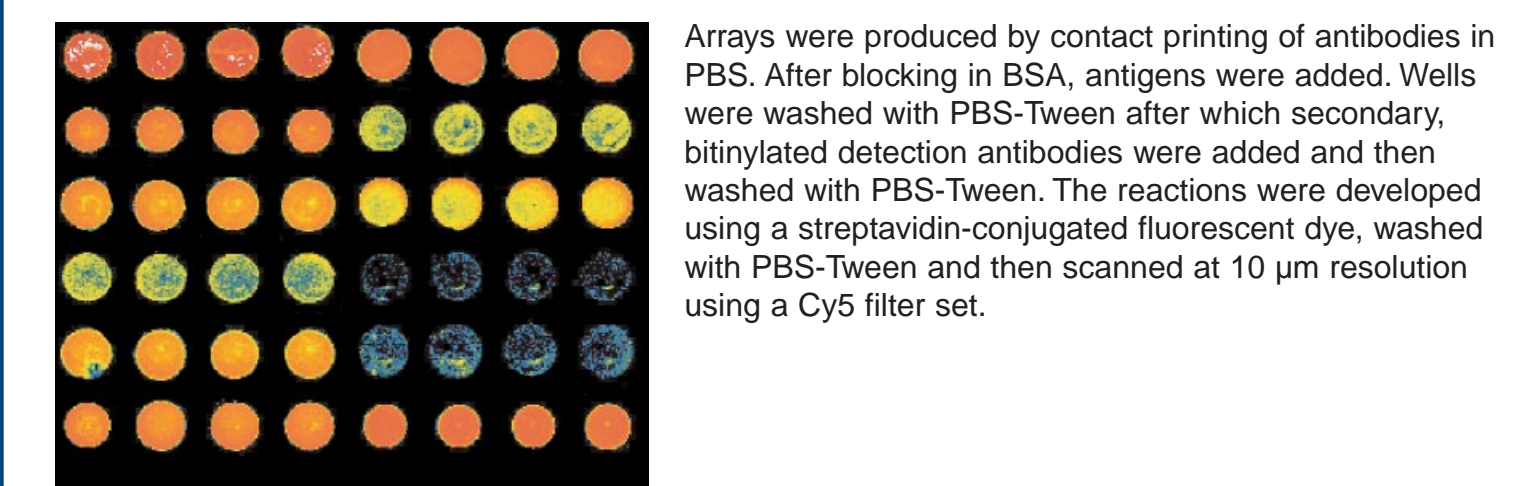
Figure 2. Spot Quality on NUNC ArrayCote APS Surface Relative to Competitor's Slides

Printing: PCR product was printed using a Pyxys system (Cartesian) to which was fitted a pin manifold holding two SMP2 Stealth Micro Spotting Pins (Arrayit).

Fixation: After printing, arrays were allowed to air dry for 1-3 hours and fixed at 254nm (8 watts, 12 cm distance) using a UV light bar for 30 seconds. Arrays may also be fixed by inversion on a UV transilluminator at 302nm for 2-3 minutes.

Prehybridization/Hybridization: After fixation, plates were blocked by prehybridization. Surfaces were exposed to a solution of 3X SSC, 0.1%SDS, and 0.1% BSA for two minutes at room temperature. Surfaces were subsequently washed in four flooding volumes of dH₂O. Before drying, surfaces were hybridized with labeled target DNA in 50% formamide, 0.1% SDS, 5X SSC. 200ng of labeled cDNA (Matrix Technologies) was transferred to a 1.5ml Microtube by four serial washes with 200µl hybridization solution for a final concentration of 0.25ng/µl. 25µl of hybridization solution was added to each well of a plate. Plates were sealed with NUNC Sealing Tape and incubated for 12-16 hours at 42°C at 100% rel. humidity.

Scanning: Plates were scanned inverted using a Tecan LS-400 Microarray Scanner at a PMT setting of 150.



Arrays were produced by contact printing of antibodies in PBS. After blocking in BSA, antigens were added. Wells were washed with PBS-Tween after which secondary, bitinylated detection antibodies were added and then washed with PBS-Tween. The reactions were developed using a streptavidin-conjugated fluorescent dye, washed with PBS-Tween and then scanned at 10 µm resolution using a Cy5 filter set.

Conclusions:

- NUNC ArrayCote surfaces demonstrate enhanced DNA binding capacity
- Excellent spot morphology is possible with PCR product arrays
- Enhanced protein binding is evident using ArrayCote surfaces
- Excellent spot quality was demonstrated in an antibody array on NUNC ArrayCote APS
- NUNC ArrayCote slides and plates are designed for multi-well arrays using standard slide and/or plate readers/scanners
- APS, aldehyde, and epoxy surface chemistries provide a range of choices for assay optimization