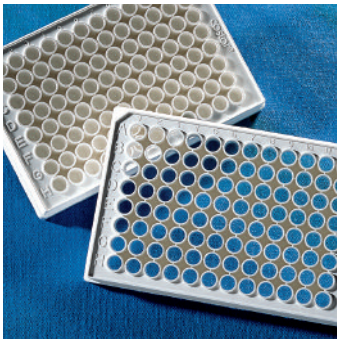
Binding Comparison of Polymer Surfaces: Introducing Corning® Nonbinding Surface (NBS™) Microplates

Application Report

Innovative Techniques in Drug Discovery



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Corning 96 well Nonbinding Surface (NBS) microplates are ideal for homogeneous assays in high-throughput screening. Studies of protein and nucleic acid binding to the NBS, when compared to polystyrene and polypropylene surfaces, provide compelling evidence of significant reduction in nonspecific binding. Here, it is demonstrated that up to a 25-fold increase in assay signal with respect to background noise can be achieved using the NBS surface for Scintillation Proximity Assay (SPA). The non-ionic, hydrophilic polymer surface is therefore well suited for enhancement of signal-to-noise ratio in these and similar assays.

Introduction

Nonspecific binding of proteins and nucleic acids to the polymer surface is an inherent problem in many homogeneous solution-based assays. The critical requirement for a novel surface technology to resolve this issue has led scientists from Corning Incorporated to develop a modified surface in the 96 well microplate format. In this report, evidence is provided that the Corning 96 well NBS microplates are superior to both conventional nontreated polystyrene as well as polypropylene microplates for conducting homogeneous assays where biomolecular binding to the solid surface is undesirable.

Methods

Protein and DNA binding to Nonbinding Surface (NBS) microplates and conventional nontreated polystyrene (PS) and polypropylene (PP) microplates were quantified by radioassay. ^{125}I-labeled IgG, BSA, and Insulin (DuPont/NEN, Boston, MA), and ^{32}P-labeled DNA-20mer-oligo and HindIII-λ DNA (DuPont/NEN, Boston, MA) was added to a solution of corresponding unlabeled protein or DNA in sodium carbonate buffer, pH 9.2, so as to achieve a concentration of 10 µg/mL of radioactive protein or DNA. Aliquots of 100 µL/well were added in triplicate of NBS, PS, and PP microplates (Corning Incorporated, Life Sciences, Lowell, MA). Following overnight incubation on a rocker platform at room temperature, the contents of each well were aspirated to waste and subsequently washed 3 times with 200 µL/well of PBS, pH 7.4. The wells were then dried and individually counted in a scintillation counter. Protein or DNA binding per well was converted to ng/cm^2 based on a surface area of 0.94 cm^2/well for 100 µL of volume.

Results and Discussion

The results shown in Table 1 indicate that the Corning Nonbinding Surface significantly reduces protein and nucleic acid binding to polymers. Additional studies show that the Nonbinding Surface effectively inhibits Madin-Darby Canine Kidney (MDCK) cell adhesion, is noncytotoxic, and is chemically stable to aqueous solutions containing 20% ethanol, 20% isopropyl alcohol, 20% dimethyl sulfoxide (DMSO), 1% sodium dodecyl sulfate (SDS), and 10M urea (unpublished results). For homogeneous assays, such as the Scintillation Proximity Assay (SPA) developed by Amersham Pharmacia Biotech, Corning NBS microplates provide for a significant reduction in nonspecific binding and consequently an increase in signal-to-noise ratio

Table 1. Protein and Nucleic Acid Binding

	Binding in ng/cm^2				
	^{125}I-IgG	^{125}I-BSA	^{125}I-Insulin	^{32}P-oligo DNA	^{32}P-λ phage DNA
Polystyrene	400	450	310	22	6
Polypropylene	380	440	370	3	<2
NBS on PS	<2.5	<2.5	5	<2	<2

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(Corning Incorporated, *The HTS Forum*, Vol. 5, Fall/Winter 1998). As demonstrated, the selection of the proper surface is critical to assay performance. The need for a nonbinding surface is essential for homogeneous assays, such as SPA, enzyme kinetics studies, fluorescence polarization studies, and for other assays in high-throughput screening that require a polymer surface which does not adsorb biomolecules in a passive, nonspecific fashion.

Conclusion

The significant reduction of nonspecific binding of proteins and nucleic acids to the Corning® NBS™ technology is accomplished by the unique modified polymer chemistry (polyethylene oxide-like) of the microplate. These attributes thus identify a superior microplate where signal-to-noise ratio is enhanced by the significant reduction of nonspecific biomolecular interaction with the surface.

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