Reactive Polystyrene (RPS) for DNA Immobilization

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Corning has demonstrated that Reactive Polystyrene (RPS) surface can be used as a solid substrate for binding amine-modified DNA molecules and subsequent hybridization. It is available as 1x8 strip plates in clear, white or black formats for colorimetric, luminescence and fluorescence applications. The surface fulfills the following criteria:

- High signal-to-noise ratio
- Rapid and specific binding of the capture probe
- Excellent binding and hybridization efficiency
- Stable bonds
- Environmentally stable
- Well-to-well reproducibility

Introduction

Nucleic acid hybridization has been used for the identification of genes for two decades. Hybridization in a microplate is a very promising method for diagnostic and high throughput screening applications due to the ease of handling microplates. So far, one of the limitations of hybridization in microplates has been the immobilization of DNA onto the surface of microplates. Clearly, the important criteria for oligonucleotide immobilization are that the coupling efficiency should be high and provide a stable linkage to the solid support.

Methods

Hybridization Assay Protocol for Colorimetric Detection of Biotinylated DNA

1. To the RPS plate, add 100 µL/well of amine modified capture probe oligonucleotide in Oligo Binding Buffer (0.1M phosphate buffer, pH 11) at a concentration of 1 pmol/well or greater. Incubate overnight at 4°C or for 1 hr. at 37°C.

2. Remove uncoupled oligonucleotide by washing the plate three times with PBS.

3. Block the unreacted active groups by adding 200 µL/well of 3% BSA in Oligo Binding Buffer. Incubate for 30 min. at 37°C and then decant.

4. Add 100 µL/well of hybridization solution (0.75M NaCl, 0.075M Sodium Citrate, 1.0% Casein, 0.1% N-lauroyl-sarcosine, 0.02% SDS) containing biotin labeled target nucleic acid at a concentration of 1 pmol/well or greater. Incubate for 1 hr. at a temperature that is 5°C (or lower) below the temperature of dissociation (Td) for the probe oligo.

   Note: Double-stranded DNA must be denatured prior to hybridizing it to the probe oligo. Denature the DNA by adding 100 µL/well of 0.25 N NaOH for 10 min. at room temperature.

5. Wash wells twice with preheated 2X SSC (0.3 M NaCl, 0.03M Sodium Citrate, pH 7.0), 0.1% SDS (Sodium Dodecyl Sulfate) and incubate for 5 min. The temperature of this solution should be the same as the hybridization temperature.

6. Add 100 µL/well of blocking solution containing Streptavidin-peroxidase conjugate diluted 1:1000. Incubate for 30 min. at 37°C.

7. Wash wells three times with PBS.

8. Add 100 µL/well of fresh substrate solution. Read the OD at 10 and 20 min.

Evaluation Parameters

- Binding efficiency
- Hybridization sensitivity
- Kinetics of capture probe
- Durability: stringent wash and different temperature storage
To assess the binding efficiency of the surfaces, add various concentrations of capture probe in oligo binding buffer while keeping the target oligo concentration the same. To assess hybridization sensitivity, use the same concentration of probe oligo while changing the target oligo concentration.

To assess binding kinetics, 1 pmol/well of capture probe in the appropriate buffer is added to 3 wells per timepoint, starting with 15 min. and timing down to 10, 5, 2.5, 2.0, 1.5, 1.0 min. and 30, 10, 5 and 2 sec. The hybridization protocol is then completed as stated.

Assessments may also be made after the initial modified capture probe oligos are bound to the surface, aspirated off, washed and then dried for 30 min. at 50°C. These assessments may include durability over time, at different temperatures and washing with detergent and high salt solution.

**Results and Discussion**

The binding efficiency using the DNA hybridization assay on RPS, DNA-BIND™ and Streptavidin** surfaces was studied. The concentration of aminated capture probe was varied from 0.05 to 10 pmol/well while the target probe concentration remained 10 pmol/well. The results (Figure 1) obtained from RPS and DNA-BIND™ surface indicate that higher signal was obtained on RPS than on DNA-BIND™ at all the concentrations of aminated oligonucleotide up to 5 pmol/well. Figure 2 is the result of the similar comparison study performed on RPS and Streptavidin surface. In this instance, biotin-labelled capture probe was used for the streptavidin surface instead of an amine-modified probe. It is noticed

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*DNA-BIND™ plates were obtained from Corning Incorporated, Cat. No. 2506.
**Streptavidin plates were purchased from Boehringer Mannheim, Cat. No. 1734776.
that when the capture probe concentration lower than 0.1 pmol/ well, the binding efficiency is higher on Streptavidin surface since there are more binding sites on the Streptavidin surface; when capture probe concentration varied from 0.1 to 10 pmol/well, higher signal was obtained on RPS. In the latter case, the more binding sites on Streptavidin surface may not be helpful for hybridization due to the steric hindrance.

Figures 3 and 4 shows the result of hybridization sensitivity studies using DNA hybridization assay on RPS, DNA-BIND™ and Streptavidin surfaces. The sensitivity test was performed by varying the concentration of target DNA from 0.01 pmol/ well to 1 pmol/well while keeping the concentration of probe DNA constant at 1 pmol/well.

In Figure 3, at all concentrations, significantly higher signal was observed on the RPS than DNA-BIND™ surface. This indicates that RPS is about 3 times more sensitive than DNA-BIND™ for DNA hybridization assays. No significant difference was found between RPS and Streptavidin surfaces (Figure 4) when these two surfaces were compared.

Figure 5 demonstrates the effect of -NH₂ and carbon-based linker arms on DNA hybridization assays. We compared 1 pmol/well of the 5' NH₂-C12-DNA with 5' NH₂-C6-DNA and 5' C9-DNA (C12-DNA is not available) with 5' DNA alone. The results indicate that the primary amine group is essential for DNA binding to both the DNA-BIND™ surface and the RPS surface. Without this group, less than 25% of the signal is obtained. The linker length does not significantly affect the DNA binding. Since there is no significant difference between the signals obtained using 5' NH₂-C12-DNA and 5' NH₂-C6-DNA, while carbon 6 linker is about the half price of carbon 12 linker, we recommend choosing either of them for different interests. This data suggests that the immobilization of aminated DNA occurs by physical and/or chemical interaction between NH₂ and the PS surfaces.

The result of binding kinetics study is shown on Figure 6. For both capture and target oligos, 1 pmol/well of concentration was used in this experiment. The immobilization took place at room temperature (25°C) since the testing was performed over a short period of time. The results suggest that capture probes can be immobilized on both surfaces within 5 sec. to reach the maximum net signal of DNA hybridization assay. This data indicates that the binding of oligonucleotides to the surfaces is very rapid.

The durability studies of bound oligo include durability over time at various temperatures and durability of stringent washing with high salt and detergent. The durability of attached DNA molecules on the microplates is necessary for optimal hybridization assays. Due to stable binding, extremely stringent conditions during hybridization are possible without loss of bound material.

![Figure 5](image5.png)

![Figure 6](image6.png)

![Figure 7](image7.png)
Figure 7 shows DNA hybridization assay results of bound oligo after storage at -20°C, 25°C and 65°C for 5 days. The results indicate that bound oligos are stable over a wide range of temperatures on both RPS and DNA-BIND™ surfaces.

The experiment of durability for stringent wash was performed by adding 1 pmol/well of capture probe to the microplates, incubate 0.5 hour at 37°C, wash off unbound DNA first with PBS for three times, then wash with high salt — 10X SSC (1.5 M NaCl, 0.15 M Sodium Citrate, pH 7.0) or detergent — 1% SDS (Sodium Dodecyl Sulfate) before blocking and hybridization. Figure 8 shows no loss of signal after stringent washing. Bound oligos are completely resistant to soaking in 1% SDS or 10X SSC for 10 min. at room temperature. No significant difference was observed between the two surfaces.

Moreover, RPS plates have been tested using several common buffer solutions (Figure 9), such as phosphate buffer, carbonate buffer, PBS and TBS. RPS worked very well in all buffer systems except the one that contains MOPS.

**Conclusion**

In this report we have presented data which suggests that RPS, a high-binding surface certified for DNA immobilization, is suitable for use as a solid support in DNA hybridization assays. Based on the results of the comparison study on commercially-available surfaces, such as a Streptavidin-coated surface and N-oxysuccinamide esters (NHS)-coated surface (DNA-BIND™), Reactive Polystyrene is the best surface for DNA immobilization and hybridization.

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