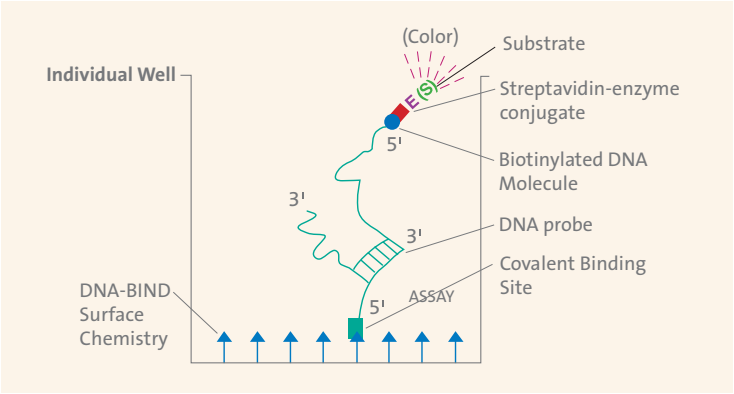
Corning® DNA-BIND® Surface

Application Guide

CORNING

CONTENTS



I. PROTOCOL FOR COLORIMETRIC DETECTION OF BIOTINYLATED DNA

1. To the Corning® DNA-BIND® microplate, add 100 µL/well of amine modified oligonucleotide (i.e., Capture Probe) in Oligo Binding Buffer (50 mM Na_2P0_4, pH 8.5, 1 mM EDTA) at a concentration of 25 pmol/well or greater. Incubate overnight at 4°C or for 1 hr at 37°C.
2. Remove uncoupled oligonucleotide by washing the microplate three times with PBS. Note: If alkaline phosphatase is used in the final step, then use maleate buffer (100 mM maleate, 150 mM NaCl, pH 7.5).
3. Block the unreacted DNA-BIND active groups by adding 200 µL of 3% BSA in Oligo Binding Buffer. Incubate for 30 min at 37°C and then decant.
4. Either DNA or RNA, which is homologous to the Capture Probe, can be hybridized to the DNA-BIND microplate. These nucleic acids must be labeled either directly or indirectly to be detected. Add 100 µL/well of hybridization solution (e.g., 5X SSC, 1.0% casien, 0.1% N-lauroylsarcosine, 0.02% sodium dodecyl sulfate) containing the target nucleic acid. For RNA transcripts, hybridize in a solution of 5X SSC, 0.05% SDS, 0.005% BSA (RNase-free BSA). Incubate 60 minutes at a temperature that is 5°C (or lower) below the temperature of dissociation (Td) for the Capture Probe.

   Note: Double-stranded DNA must be denatured prior to hybridizing it to the Capture Probe. Denature the DNA by boiling for 5 minutes in a water bath. Quickly transfer the tube to ice or flash freeze in a dry ice-ethanol bath. Inadequate denaturing or slow cooling may result in poorly denatured DNA, and result in poor hybridization efficiency (i.e., as measured by low signal).
5. Wash wells with preheated 2X SSC, 0.1% SDS twice and soak for 5 minutes. The temperature of this solution should be the same as the hybridization temperature.
6. Add 200 µL/well of blocking solution. Incubate for 30 minutes at 37°C and then decant. (This step can be omitted if strong signals are usually detected.)
7. Add 100 µL of blocking solution containing Streptavidin-alkaline phosphatase or Streptavidin-peroxidase conjugate diluted 1:1000 (i.e., 10 µL into 10 mL blocking solution). Incubate for 30 minutes at 37°C.
8. Wash wells three times with PBS (or maleate buffer, if alkaline phosphatase is used).
9. Prepare fresh substrate solution and add 100 µL/well. Read the OD at 37°C at 0, 10, 20, 30, 40, 50, and 60 minutes.

II. INTRODUCTION: CHEMISTRY OF CORNING® DNA-BIND®

Corning DNA-BIND 96 well microplates and 1 x 8 Stripwell™ microplates are specifically designed for the immobilization of aminated DNA for use in immuno-PCR and other nucleic acid hybridization assays. DNA-BIND surface chemistry enables covalent attachment of aminated nucleic acids to the surface of the microplate.

The surface of the DNA-BIND microplate is coated with a layer of reactive N-oxysuccinimide esters, referred to as NOS groups, which react with nucleophiles such as primary amines. These NOS groups are covalently linked to the polystyrene surface and thus cannot be washed off the microplate. NOS is a widely applied active ester commonly used to couple NOS-activated molecules to protein. DNA with primary amines added synthetically or by *in vitro* manipulation can be directly coupled to the NOS surface. This coupling is specific and not affected by the amines attached to the adenine, guanine, and cytosine rings. Within the bases, the electron resonance created by the ring stabilizes the amino groups making them relatively unreactive as compared to the attached primary amines. In fact, nucleic acids lack groups that will react with NOS, thus DNA and RNA can be considered inert biomolecules during standard coupling procedures.

When primary amines react with NOS groups at a slightly alkaline pH, the ester undergoes nucleophilic substitution as the amine attacks the carbonyl group and displaces the N-oxysuccinimide group (Fig. 1). The specificity of these reactions allows for a very specific coupling of DNA to DNA-BIND microplates.

Several mechanisms are available for adding a primary amine onto DNA. The most common method is to incorporate the amine onto either the 5' or 3' end of the molecule during the synthesis. This amine is usually attached to the phosphoribose backbone via a carbon linker of either three, six, or twelve carbons. This linker is useful in extending the oligonucleotide away from the microplate surface, thus allowing greater access to homologous nucleic acids in solution.

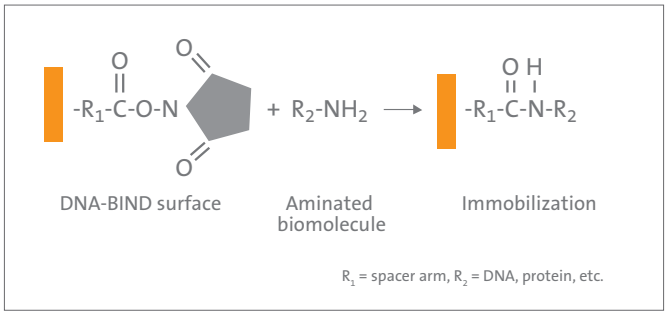


Figure 1. Coupling of DNA to Corning DNA-BIND microplate.

The actual coupling of an aminated oligonucleotide to Corning® DNA-BIND® is accomplished by mixing the DNA in a phosphate buffer, pH 8.5, and then adding the solution to a DNA-BIND-coated well. Unlike other chemistries, NOS is an activated ester and can be used for coupling without the addition of diimide coupling reagents, such as EDC. The coupling event with the DNA-BIND surface occurs very rapidly. Kinetic analysis demonstrates that covalent linkage occurs predominantly within the first minute of the reaction at 37°C. Subsequent incubation allows for a non-covalent absorption of oligonucleotides to the NOS surface. This non-covalent interaction is specifically related to the presence and length of the carbon linkers, which are added during the amination step of the oligonucleotide synthesis. Generally, non-aminated DNA with no carbon linker does not adsorb to the DNA-BIND surface resulting in low background signals. Aminated DNA, which is non-covalently bound to the microplate, can be removed by a post-coupling wash with a SDS based wash solution, e.g., 1% SDS, 100 mM maleate buffer, pH 7.5, 150 mM NaCl.

The covalent linkage of the DNA to the DNA-BIND surface can be applied to a variety of assay systems. The specificity of homologous strands of DNA has allowed for the detection of nucleic acids with specifically labeled probes since the onset of recombinant DNA technology in the 1970s. Southern† (1975) initially developed the DNA blot which has since been expanded and modified. These approaches to nucleic acid detection rely on immobilizing the DNA/RNA and then hybridizing a homologous labeled probe to the immobilized molecule. A modification of the Southern blot is the reverse dot blot. In this technique, oligonucleotides are immobilized on a surface and are used to capture larger DNA molecules. DNA-BIND microplates can be used for both hybridization formats.

III. ASSAY DESIGN

Many strategies have been devised for 96 well microplate-based DNA probe assays. In all options, (i) a nucleic acid molecule is attached to the microplate surface, (ii) a second nucleic acid molecule in hybridization solution is hybridized to the immobilized molecule, and (iii) the second nucleic acid is measured either directly or indirectly.

A relatively simple assay involves coupling an aminated oligonucleotide to the DNA-BIND microplate followed by hybridization of denatured PCR product to the immobilized oligonucleotide. Detection of the PCR product could be accomplished several ways, including the incorporation of biotinylated dCTP into the molecule during PCR. This labeled molecule could then be detected with a standard Streptavidin detection system.

†Southern, E. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503-517.

A second assay strategy could involve covalently binding an oligonucleotide to the Corning® DNA-BIND® microplate, and then hybridizing an isolated RNA transcript to the oligonucleotide. A second biotinylated oligonucleotide could then be hybridized to the RNA. In this strategy, the objective would be to detect or measure RNA, as for an assay to measure viable bacteria in food.

IV. OPTIMIZATION OF DNA PROBE ASSAYS

This guide is generalized based on conditions assessed at Corning for a particular set of capture probe and biotinylated target DNA. However, experience shows different sets of capture and target nucleic acids will behave differently and conditions may require optimization. The following data demonstrate parameters assessed and may provide insight to researchers planning to optimize their own assays.

A. Oligonucleotide Coupling

A hybridization assay requires the covalent attachment of an oligonucleotide to the DNA-BIND microplate. This involves (i) coupling the oligonucleotide to the NOS surface, (ii) washing the microplate to remove uncoupled oligonucleotides, and (iii) inactivating the remaining NOS groups by reacting with a second molecule containing a primary amino group.

Numerous parameters affect the coupling of an aminated oligonucleotide to the DNA-BIND microplate. These parameters are pH, oligonucleotide concentration, temperature of coupling, oligonucleotide purity, and time of coupling. Though not immediately important to the preparation of the microplate, the length of the aminated carbon linker arm on the oligo greatly affects the assay.

1. Buffers for Coupling

Aminated DNA will react with the DNA-BIND surface over a wide pH range. The most important characteristic of the buffer used for coupling is that it does not possess a primary amine, e.g., TRIS. Such primary amines will compete with the aminated oligonucleotide for the NOS group on the DNA-BIND surface. The optimal coupling reaction occurs at pH 8.5 in 50 mM phosphate, 1 mM EDTA. Phosphate buffers are suitable for coupling at pHs 7 to 8.5 and 9.5 to 11. For a lower pH, citrate may be used between pH 4 to 6. Borate buffer should not be used for coupling at pH 9 due to poor coupling efficiency.

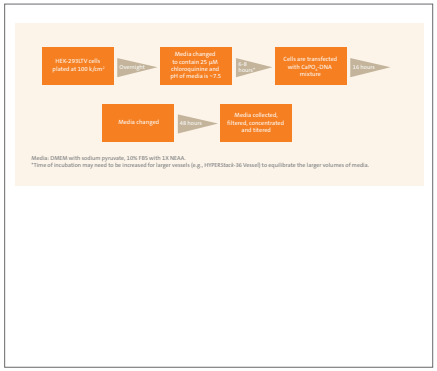


Figure 2. Capture Probe Coupling

2. Kinetics of Coupling

The coupling of aminated DNA to a Corning® DNA-BIND® surface is a very rapid event, which can be influenced by the concentration of the aminated oligonucleotide and the reaction temperature. When aqueous aminated oligonucleotides are added to a DNA-BIND-coated well, numerous nucleophilic reactions with the NOS groups can occur. The displacement of the primary amine for the NOS group is the predominant reaction, but water and nucleotide base amines will also react with NOS at a much slower rate. Increasing the temperature of the reaction will increase the rate of the main coupling reaction, but it may also accelerate the less desirable side reactions. Though higher incubation temperatures will speed up the coupling reaction, the efficiency of the desired reaction may decrease as less reactive nucleophiles begin to compete with the aminated oligonucleotide for the limited number of NOS groups. In general, oligonucleotide coupling is most efficient at 4°C and decreases as the temperature increases. The rate at which aminated oligonucleotides couple to the DNA-BIND can also be influenced by changing the concentration of oligonucleotide during the coupling reaction. At low oligonucleotide concentrations, i.e., <25 pmoles/100 µL, the rate of coupling can be substantially lower than at concentrations of 100 pmoles/well or higher. To attain rapid coupling of the oligonucleotide to the DNA-BIND surface, excessive concentrations of aminated oligonucleotide should be used. The concentration needed to obtain sufficient immobilized oligonucleotide will depend upon the size of the molecule, reaction temperature, and the duration of the reaction. In general, an overnight reaction at 4°C leads to efficient coupling, though not all of the retained oligonucleotide is covalently bound. Though nucleic acids generally do not adsorb to the DNA-BIND microplate, the aminated carbon linker arm can adsorb as well as covalently attach. Washing the DNA-BIND surface with a SDS solution can aid in the removal of adsorbed capture probes.

3. Effect of Oligo Purity

Synthetic oligonucleotides can be prepared to different degrees of purity. Obviously, the greater the purity of the preparation, the lower the yield and greater the cost. Many surface chemistries require high purity oligonucleotides for the preparation of 96 well microplates used for nucleic acid hybridization assays. The necessity of high purity oligonucleotides relates directly to the chemistry of the microplate surface and coupling reaction. The major impurities are incomplete sequences, i.e., truncated oligonucleotides. These molecules are shorter than the full length product and are usually caused by excess moisture. During each round of synthesis, a small percentage of the growing oligonucleotides are truncated. The longer the complete oligonucleotide, the greater percentage become truncated during synthesis. In very long oligonucleotides, e.g., >50 bases, the amount of truncated impurities can equal the full length product. Shorter truncated molecules are incapable of coupling to the amino carbon linker amidite due to the capping step during synthesis. This characteristic can be exploited for assays which use Corning® DNA-BIND® microplates.

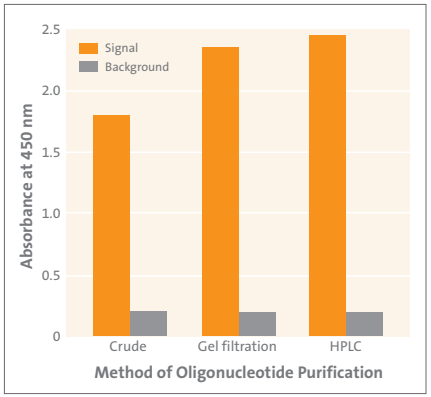


Figure 3. Effect of Post-synthetic Purification Method on Signal Strength and Background

Oligonucleotides containing a 5' amino carbon linker are highly specific for the DNA-BIND surface over the truncated oligonucleotides. The surface of the DNA-BIND microplate does not actively or passively react with truncated oligonucleotides. Alternatively, the NOS groups overwhelmingly react with the primary amines, thus making the coupling specific for full length molecules. For this reason, overly pure oligonucleotides are not required for DNA-BIND microplates. In fact, the signals produced from crude, gel filtration, and reverse phase purified oligonucleotides were found to be equivalent, while background remained relatively low. Oligonucleotides can also be synthesized with a 3' amino carbon linker, and unlike 5'-modified counterparts, these derivatives will couple truncated molecules to the NOS surface.

4. Effect of Linker Arm Length

Oligonucleotides attached to DNA-BIND microplates require a carbon linker to extend the capture probe away from the microplate towards the proximity of the target molecules. Several different linkers are available, including amino C3, C5, C6, and C12. Though more expensive, larger linkers can also be constructed by using specialized carbon linker amidites. Generally, amino C12 linkers have been found to repeatedly provide the strongest signal for DNA assays. Alternatively, amino C6 linkers can be used without significant loss of signal. The amino C6 linkers are generally much less expensive than amino C12 or longer combinations. However, amino C3 typically yield poor signal and should be avoided.

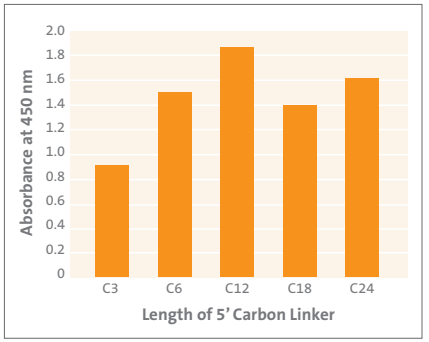


Figure 4. Effect of Oligonucleotide Amino Linker on Signal Strength

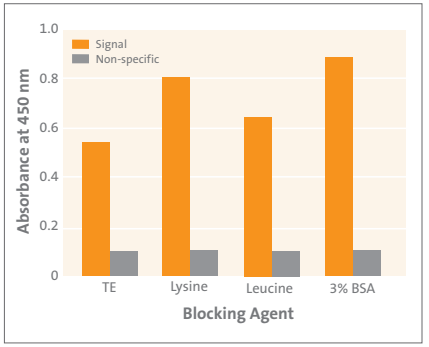


Figure 5. Comparison of Post Coupling Blocking Reagents

B. Post Coupling Blocking

Following the coupling of the capture probe to the Corning® DNA-BIND® microplate, the remaining NOS groups should be inactivated. The easiest route to accomplish this inactivation is to simply add a high concentration of molecules containing reactive amino groups to the well, followed by an incubation. Several different blocking reagents have been assessed for inactivating NOS, and these include TE buffer (10 mM Tris, pH 8, 1 mM EDTA), 10 mM lysine (in 50 mM phosphate, pH 8.5), 10 mM leucine (in 50 mM phosphate, pH 8.5), and 3% BSA (in 50 mM phosphate, pH 8.5). Of these, BSA blocks without hindering a strong signal. Background for all four blocking agents is relatively low (i.e., <0.1).

C. Hybridization and Detection

1. Nucleic Acid Hybridization

The sequence of steps involved in a 96 well microplate based DNA assay is very similar to those of a DNA blot. However, due to the surface chemistry of the DNA-BIND microplate and the extension of the oligonucleotide capture probe into the well, several parameters are different from the traditional blot. Following the coupling of the capture probe to the microplate, the subsequent logical step would be to prehybridize the wells to prevent non-specific absorption of the target DNA. However, since DNA lacking amino carbon linkers do not adsorb to DNA-BIND microplates, prehybridization is actually detrimental to the assay. A standard prehybridization solution (e.g., 5X SSC, 1.0% powdered dry milk, 0.1% N-lauroylsarcosine, 0.02% SDS) significantly lowers signal strength. Omission of this step does not result in significant increases in background.

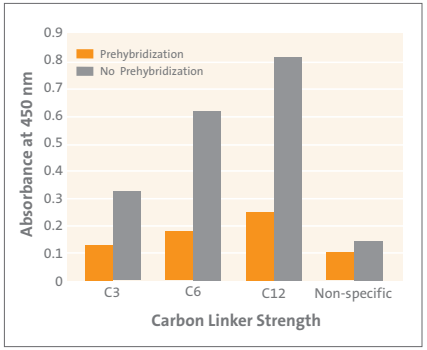


Figure 6. Effect of Prehybridization on Signal Strength

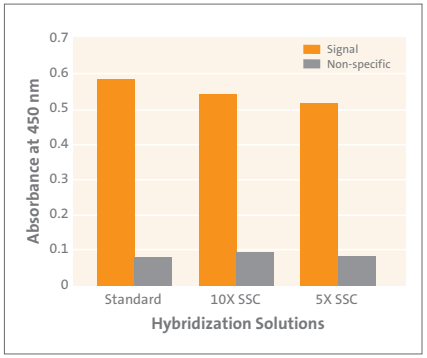


Figure 7. Effect of Hybridization Solution on Signal Strength

The hybridization of target nucleic acid to the capture probe follows many of the traditional rules applied to using oligonucleotides for probes and primers. Generally, hybridization of the target nucleic acid should occur slightly below the temperature at which the capture probe will dissociate (or melt) from the target molecule. A common estimate of the temperature of disassociation (T_d) for an oligonucleotide is based on the number of A, T, G, and Cs and is calculated by the following formula:

$$T_d = 2 \times (\text{number of A+T}) + 4 \times (\text{number of G+C})$$

This formula can generally be applied for oligonucleotides between 11 and 23 bases. For instance, an 18mer which has eight A + T and ten G + C would have a T_d of 56°C as calculated by this formula. An accepted rule for hybridization, especially applied to PCR, is that the temperature of hybridization should be T_d - 5°C.

The composition of the hybridization solution can affect the strength of the assay. Several hybridization solutions have been assessed for both DNA and RNA hybridization, and significant differences exist between the two molecules. For DNA, a standard hybridization solution of 5X SSC, 1.0% casien, 0.1% N-lauroylsarcosine, 0.02% sodium dodecyl sulfate was compared to 10X SSC and 5X SSC. All three solutions worked relatively well, though the standard solution did generate slightly higher signals.

The hybridization of RNA to immobilized oligonucleotides requires a different hybridization solution than a DNA assay. A biotinylated RNA transcript exhibited very different hybridization behavior as compared to the DNA. Most notable was the lack of signal when the RNA was hybridized in the standard hybridization solution and the extreme background associated with hybridization in 5X SSC. The hybridization solution which produced the best signal to noise ratio while retaining specificity was a 5X SSC, 0.05% SDS solution. The negative effect of protein on the RNA hybridization may be attributed to RNase, which is often a contaminant of many protein preparations.

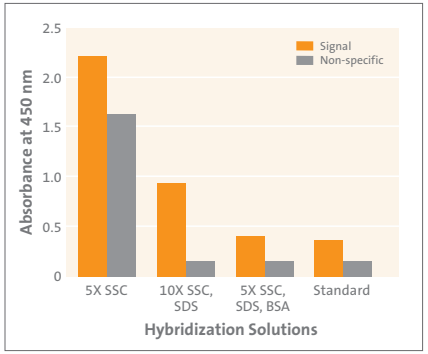


Figure 8. Effect of RNA Hybridization Solution on Signal Strength and Background

2. Preparation of Target Nucleic Acids

Most assays designed to hybridize DNA typically target PCR products. By incorporating a label into the PCR product, the hybridized DNA can easily be detected, such as the detection of biotinylated DNA with Streptavidin-enzyme conjugates. Like other blotting strategies, the two strands of the PCR product must by separated, i.e., denatured, to allow their hybridization to the capture probe. This denaturation can be done either by heating or with chemical denaturation. The most common method of denaturing DNA is to heat the PCR product by boiling in a tightly capped tube. The PCR product is diluted in hybridization solution and then denatured in a beaker of boiling water for 10 minutes. The denatured DNA

should be chilled rapidly following boiling in order to prevent the homologous strands from annealing. The solution should be flash frozen in a dry ice/ethanol bath. Alternately, it can be immersed in ice.

Detecting PCR products can sometimes be more difficult than expected. The concentration of DNA in a PCR reaction is extremely high and simple denaturation of the solution can often be followed by rapid annealing of the homologous strands. If this annealing is unpreventable, then inadequate hybridization to the microplate may result in a low signal. When designing an assay, it is often necessary to empirically determine the best concentration of PCR product to use. A balance between too diluted and too concentrated must be determined in order to attain a good signal.

3. Effect of Time, Temperature, and Salt on Hybridization

In blotting, the hybridization of nucleic acid probes to the membrane-bound target molecule is normally the longest incubation of the procedure. In Southern blotting, the hybridization step can require 16 hours or more. Fortunately, the rate of capture of target molecules in a 96 well microplate format is much faster than with standard membrane blots. This can be attributed to the high concentration of capture probe, extension of the capture probe away from the surface, and the small volume of hybridization solution as compared to the area of the Corning® DNA-BIND®-coated well. In general, the hybridization of DNA and RNA to the capture probe requires 1 hour.

The temperature of hybridization is also very important and may reflect either assay stringency or strategy. The hybridization temperature must be below the temperature of dissociation (T_d) for the capture probe. As noted previously, Td can be estimated for oliognucleotides between 11 and 23 bases as T_d = [2 x (A+T)] + [4 x (G+C)]. For high stringency assays, the hybridization temperature should be below T_d, e.g., Td - 5°C. This same temperature should also be used for post hybridization washing. However, substantially lower temperatures can also be used in order to increase the rate of hybridization. If lower temperatures are used, the stringency of hybridization is provided by high temperature post-hybridization washes.

The concentration of salt can greatly affect the hybridization. Monovalent cations stabilize the double helix and can be used to aid in the hybridization process. Normally, hybridization solutions use 5X SCC (diluted from 20x SSC which is 0.3 M Na citrate, pH 7, 3 M NaCl). Lowering the concentration of sodium ions reduces the hybridization rate while increasing the stringency.

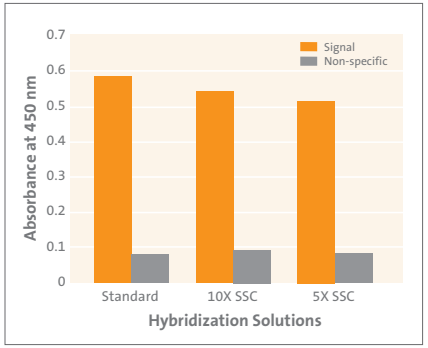


Figure 9. Effect of Hybridization Solution on Signal Strength

For DNA probe assays, theory is often difficult to apply to practice. The conditions for hybridization, which include temperature and time, should be tested and optimized for each PCR product assayed.

D. Post Hybridization Washing

Post-hybridization washing is necessary to remove unbound and non-specifically hybridized DNA from the Corning® DNA-BIND®-coated well. Post-hybridization wash solutions are usually formulated from stocks of 20X SSC (0.3 M Na citrate, pH 7, 3 M NaCl) and 10% SDS solution. These solutions are heated to a temperature that will allow for the dissociation of poorly hybridized molecules. By combining heat with higher dilutions of the 20X SSC, the stringency of the system is increased.

Common post-hybridization wash solutions include 2X SSC, 0.5X SSC, and 0.2X SSC, all with 0.1% SDS solution. These solutions are heated and used to repeatedly rinse the DNA-BIND-coated well. Additionally, it is common to flood a well with wash solution, followed by incubating the microplate at an elevated temperature.

E. Detection

The detection of biotinylated target DNA is very similar to an ELISA. Following the post-hybridization wash, a solution of protein is used to block the remaining active sites prior to the addition of a Streptavidin-enzyme conjugate. Blocking solutions are composed of protein, usually BSA, casien, or powdered dry milk, in a buffer (e.g., 3% BSA in TBS, Tris Buffered Saline). For DNA-BIND microplates, a blocking solution of 3% BSA in maleate buffer (100 maleate, pH 7.5, 150 mM NaCl) is used for a 30-minute blocking step. However, microplates in which the NOS groups were inactivated with BSA may not require post-hybridization blocking. The omission of blocking does not necessarily lead to elevated background or decreased signal-to-noise ratios.

The addition of Streptavidin-enzyme conjugate follows blocking. The enzyme conjugate is usually alkaline phosphatase or peroxidase. The stock Streptavidin enzyme conjugate is diluted into blocking solution and then added to the well for 30 minutes. Longer incubations are usually not necessary due to the high affinity of Streptavidin for biotin. The efficacy of the Streptavidin-enzyme conjugate will vary between manufacturers, and the optimal dilution for each will need to be determined empirically. Generally, dilutions will range from 1:500 to 1:10,000.

Following the binding of the Streptavidin-enzyme conjugate, the microplate is washed to remove excess reagent. Wash buffers may contain a mild detergent, such as Tween 20, in TBS or PBS. Three washes will usually remove most non-specifically adsorbed material. DNA-BIND microplates typically have low non-specific adsorption, however, thorough washing is still recommended.

The final reagent added to the assay is the enzyme substrate. For alkaline phosphatase, p-nitrophenyl phosphate may be used, and for peroxidase, ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] is suggested. These substrates can be purchased in ready to use tablets compounded with buffer and are simply added to water for use. Following the addition of substrate, the optical density of the Corning® DNA-BIND®-coated wells are read at 450 nm on a 96 well microplate reader. Signals should be above 0.6 while background should be well below 0.1.

F. Quantitation

DNA can be quantitated within a limited range. To quantitate a PCR product or other labeled nucleic acid, a standard curve must be prepared. A convenient curve can be constructed by hybridizing a range of concentrations of a biotinylated antisense oligonucleotide to the capture probe. This control molecule is recommended since it can be produced in large quantity, e.g., up to 10 µmoles or greater, and stored in frozen aliquots for future use.

Generally, the linearity of DNA hybridization assays cover is limited to a range of one magnitude when using colorimetric detection. By changing the detection strategy to fluorescence or luminometry, the range may be extended.

V. SUMMARY

DNA-BIND microplates provide researchers with a powerful tool for nucleic acid hybridization assays in a format synonymous with common enzyme immunoassays. The N-oxysuccinimide groups efficiently react with oligonucleotides possessing amino carbon linker arms, forming a covalently attached capture probe. The oligonucleotides are best derivatized at the 5' end with an amino linker containing a C12 carbon chain. These oligonucleotides can both covalently attach to the microplate and adsorb via the carbon linker. Native DNA does not readily adsorb to the DNA-BIND surface, a property which aids in the reduction of background signal. Hybridization of labeled target DNA can be performed in a simple hybridization buffer within an hour. Wells are subsequently washed, blocked, and then incubated with an enzyme conjugate specific for the label. The enzyme conjugate is washed, substrate is added, and the presence of target DNA is determined based on a positive colorimetric assay.

VI. TROUBLESHOOTING GUIDE

The following guide is provided in order to indicate where potential problems may occur while performing a DNA-based 96 well microplate assay.

Problem	Cause	Solution
Lack of Signal	No Target	Check source or reaction
	Inadequate denaturation of PCR product and then flash freezing	Denature PCR product by boiling
	No capture probe on the microplate	Check pH of Oligo Binding Buffer. Use non-aminated buffers.
		Check quality of probe; resynthesize
		Old open microplates can lose NOS groups by reaction with water. Do not open microplates until ready to use.
		Insufficient coupling due to short incubation. Increase incubation time.
	Capture probe is non-specific	Evaluate sequences to ensure the correct capture probe sequence is being used.
Low Signal	Capture probe may have secondary structure or homology with itself.	Redesign capture probe or lower concentration of probe on the microplate.
	Target DNA may have secondary structure which prevents hybridization to the capture probe.	Redesign PCR reaction or pretreat target with DNA-modifying enzymes.
	Failed detection system.	Enzyme-conjugate may be bad; test with a dot blot and if necessary, replace.
		Substrate may be bad; test and if necessary, replace.
High Background	Non-specific or degenerated capture probe.	Redesign the capture probe to increase specificity.
	Insufficient blocking of microplate.	Omission of blocking step. Include blocking at indicated points of the assay.

VII. OTHER APPLICATIONS

The Corning® DNA-BIND® surface has also proved useful for applications other than immobilization of ssDNA. These applications include the immobilization of small peptides, enzymes, and small antigens that denature and become inactive when passively adsorbed to a surface. Below is a protocol to be used for the immobilization of such molecules:

1. Dilute biomolecule possessing an available amine group to a concentration compatible with the size of the molecule (the smaller the size, the higher the concentration). Example: 500 to 5,000 daltons: 1 to 10 mg/mL. PBS pH 9.0 has been shown to be a suitable buffer.
2. Add 100 µL/well of sample and incubate 1 hour at room temperature.
3. Decant and rinse three times with an appropriate wash buffer containing detergent. (PBS and Imidizole with Tween 20 have been shown to be adequate wash buffers.)
4. Block the remaining active sites with 2% BSA. Incubate 30 minutes at room temperature.
5. Decant the solution. Do not rinse.
6. Dilute subsequent reagents in an appropriate buffer containing 10% normal serum (e.g., PBS pH 7.4 with 10% fetal bovine serum). The addition of 10% normal serum to this diluent has been shown to reduce non-specific background binding.
7. Proceed with remainder of assay.

ADDITIONAL LITERATURE AVAILABLE ONLINE:

- **Comprehensive DNA Probe Assay Bibliography**
  http://catalog2.corning.com/Lifesciences/media/pdf/t_compdnaprobebibl.pdf
- **Technical Parameters for the Use of Corning® DNA-BIND® Products in High-Throughput Screening**
  http://catalog2.corning.com/Lifesciences/media/pdf/an_techpara_dnabind.pdf

For more information, call Corning Technical Service at 1.800.492.1110.

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