Application Note

Thermo Scientific Nunc Streptavidin Coated Plates for Molecular Biology

Key Words

Streptavidin coated, coated plates, PCR-ELISA, capture oligonucleotide, DNA hybridization.

Goal

The goal of this tech note is to detect the binding of capture oligonucleotides to Thermo Scientific™ Nunc™ Streptavidin coated plates when performing a DNA hybridization assay. Following to estimate the amount of PCR products that can be hybridized and detected.

Nunc Streptavidin coated plates are ideal for binding of small biotinylated molecules and therefore have several applications in molecular biology as well as in immunology. In this Tech Note we have compared the Streptavidin coated plates with two different types of Streptavidin coated plates from leading manufacturers by performing a DNA hybridization assay and a PCR-ELISA.

DNA hybridization assay

DNA hybridization assays were performed in order to measure the capacity of the Streptavidin coated plates to bind biotinylated oligonucleotides.

A competitive assay was performed. 100 nm biotinylated capture oligonucleotide and varying concentrations of free biotin were added to the surface of the Streptavidin coated plates. Captured oligonucleotide was detected by hybridization of a fluorescein labeled oligonucleotide detection probe. The Nunc Streptavidin coated plates were tested against two Streptavidin coated plates from leading manufacturers (Fig. 1). The Nunc Streptavidin coated plates have a sensitivity as good as or better than the plates from leading manufacturers.

PCR-ELISA

To estimate the amount of PCR product that can be hybridized to the Nunc Streptavidin coated plates a PCR-ELISA was performed. A serial dilution of a digoxigenin labeled PCR-amplified product was added to the wells, and the amount of hybridized PCR product was detected with anti-digoxigenin conjugated alkaline phosphatase. As seen in Fig. 2, Nunc Streptavidin coated plates have a sensitivity similarly to the two other plates tested. It is possible to detect 0.45 ng PCR products, corresponding to 2.3 x 10^{-15} moles. The sensitivity of the Streptavidin coated plates is 1:10 in comparison to detection of PCR amplicons by gel electrophoresis. Furthermore, the applications of ELISA methods make it easier to analyse a larger number of samples.

Methods

Binding of capture oligonucleotides to Streptavidin plates

The capture oligonucleotide has a biotin group and a spacer of 10 Thymidine in the 5' end. 100 µL of a coating mix containing 100 nm capture oligonucleotide and varying known amounts of free biotin (50,000 nm - 0 nm) in 100 mm Tris-HCl (pH 7.5), 150 mm NaCl and 0.1% Tween 20 were added to each well. The wells were sealed and incubated at 37°C for one hour. The wells were washed three times, soaked for five minutes and washed three times, all with 100 mm Tris-HCl (pH 7.5), 150 mm NaCl, and 0.1% Tween 20 at room temperature.

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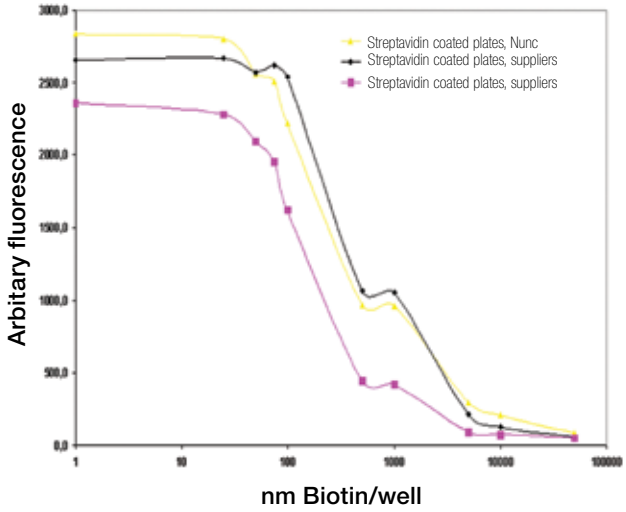

Fig. 1. DNA hybridization assay
The wells were coated with 100 nm biotinylated oligonucleotide and varying concentrations of free biotin. The amount of captured biotinylated oligonucleotide was measured by hybridization with a fluorescein labeled detection probe.

The amount of hybridized DNA is given as arbitrary fluorescence signal.

A background fluorescence level, due to unspecific binding of antibody was for all the tested plates lower than 2.5% (results not shown).

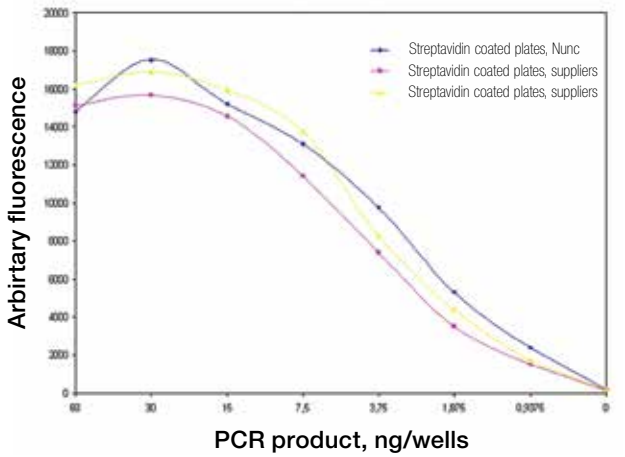

Fig. 2. PCR-ELISA
Detection of PCR products on Streptavidin coated plates. Denatured digoxiginin labeled PCR product was captured by a probe immobilized to the Streptavidin coated plates and detected by anti-digoxiginin conjugated alkaline phophatase. The detection level is given as arbitrary fluorescence signal.

Hybridization

A solution of 0.25 nm 5' fluorescein labeled detection probe and 50 nm unlabeled detection probe in 100 µL hybridization buffer (5 x SSC, 0.1% Tween 20, 0.5% blocking reagent) were added to each well and samples hybridized for one hour at 50°C. After hybridization, the wells were washed three times, soaked for 15 minutes and washed three times, all with 0.5 x SSC, 0.1% Tween 20. Anti-fluorescein conjugated alkaline phosphatase was diluted 500 times in 100 mm Tris-HCl (pH 7.5), 150 mm NaCl, 0.1% Tween 20, 0.5% blocking reagent and 100 µL were added to each well. The wells were incubated for one hour at 50°C and then washed three times, soaked for five minutes and washed three times, all with 100 mm Tris-HCl (pH 7.5), 150 mm NaCl, and 0.1% Tween 20 at room temperature. 100 µL substrate solution (1 mm 4-methylumbelliferyl phosphate dissolved in 1 M diethanolamine (pH 9.8) and 1 mm $MgCl_2$) were added, and the enzyme reaction was carried out for 30 minutes at 50°C. The fluorescence signal was read on a fluorescence plate reader: excitation 355 nm, emission 450 nm.

PCR amplification and detection

Amplification reactions were performed in a total volume of 50 µL containing buffer (10 mm Tris-HCl (pH 8.3), 50 mm KCl, 0.1% Tween 20), 2.5 mm $MgCl_2$, 1 mg/mL BSA, 4 µm DIG-11-dUTP, 0.1 mm dTTP, 0.2 mm of dATP, dCTP and dGTP, respectively, 0.5 µm of each primer, 1 U Taq DNA polymerase and DNA template (10^{-15} moles). The PCR cycle was: 94°C for 10 minutes, cycle 1-35: 94°C for 25 seconds, 55°C for 25 seconds, 72°C for 25 seconds, followed by one cycle at 72°C for 10 minutes. The PCR amplification product was analysed on a 1% agarose gel and quantitated. A serial dilution of the amplification product was performed, and 10 µL were denatured with 10 µL 1 M NaOH. Denatured PCR product was transferred to Streptavidin coated plates, containing 80 µL of 6.25 x SSC, 0.625% blocking reagent, 0.125% Tween 20 and 0.5 M NaH_2PO_4 (pH 6.5). The wells were incubated for one hour at 50°C and then washed three times, soaked for 15 minutes at 50°C, washed three times all with 0.5 x SSC and 0.1% Tween 20 at room temperature. To each well 100 µL anti-digoxigenin conjugated alkaline phosphatase diluted 1:500 in 100 mm Tris-HCl (pH 7.5), 150 mm NaCl, 0.1% Tween 20 and 0.5% blocking reagent was added. The rest of the detection procedure were the same as described under hybridization.

Conclusion

Using the Nunc Streptavidin coated plates it is possible to detect 0.45 ng PCR products, corresponding to 2.3 x 10^{-15} moles.

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