Thermo Scientific Nunc NucleoLink versus CovaLink Surfaces

Application Note

Key Words

Thermo Scientific™ Nunc™ CovaLink™, Thermo Scientific™ Nunc™ NucleoLink™, Thermo Scientific™ Nunc™ Immuno Washer, binding of oligonucleotides, ELISA, Detection of Immobilized Amplified Product in a One Phase System (DIAPOPS), DNA hybridization.

Goal

The goal of this application note is to highlight variations between binding capabilities and applications on Nunc NucloLink and Nunc Covalink surfaces.

When performing ELISA-like procedures with nucleic acids in Nunc MicroWell plates, the adsorption of nucleic acid molecules to the plastic surface is one factor which distinguishes success from failure.

We provide two types of surfaces for covalent immobilisation of nucleic acids: Nunc CovaLink and Nunc NucleoLink.

By a process, the Nunc CovaLink surface is grafted covalently to the polymer surface with a linker molecule ending in a secondary amino group. Nucleic acid molecules are bound at only the 5'-end to this linker molecule by carbodiimide condensation.

The Nunc NucleoLink surface binds nucleic acids by carbodiimide condensation using a procedure similar to CovaLink. NucleoLink binds more nucleic acids than CovaLink, and more importantly, the NucleoLink surface is heat stable at temperatures up to 120°C. Combined with the thin wall (0.35 mm) this makes NucleoLink highly suitable for solid phase PCR.

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Binding of oligonucleotide to NucleoLink and CovaLink

Various concentrations of ^{32}P labeled oligonucleotide (0.133 ng/µL; 0.266 ng/µL; 0.4 ng/µL; 13.33 ng/µL) in 10 mm 1-methyl imidazole were pipetted into NucleoLink or CovaLink Strips, 75 µL/well. 25 µL freshly made 40 mm (EDC) in 10 mm 1-methyl imidazole was added to each well containing oligonucleotide. The CovaLink and NucleoLink Strips were then sealed and incubated at 50˚C for five hours.

After incubation the strips were washed with 0.4 N NaOH, 0.25% Tween 20 using a Thermo Scientific Nunc Immuno Washer. The strips were washed three times, incubated at 50˚C for 15 minutes and finally re-washed three times. The amount of covalently bound DNA was measured by liquid scintillation.

Conclusion

20% to 50% of the oligonucleotide present during coating is bound to NucleoLink.

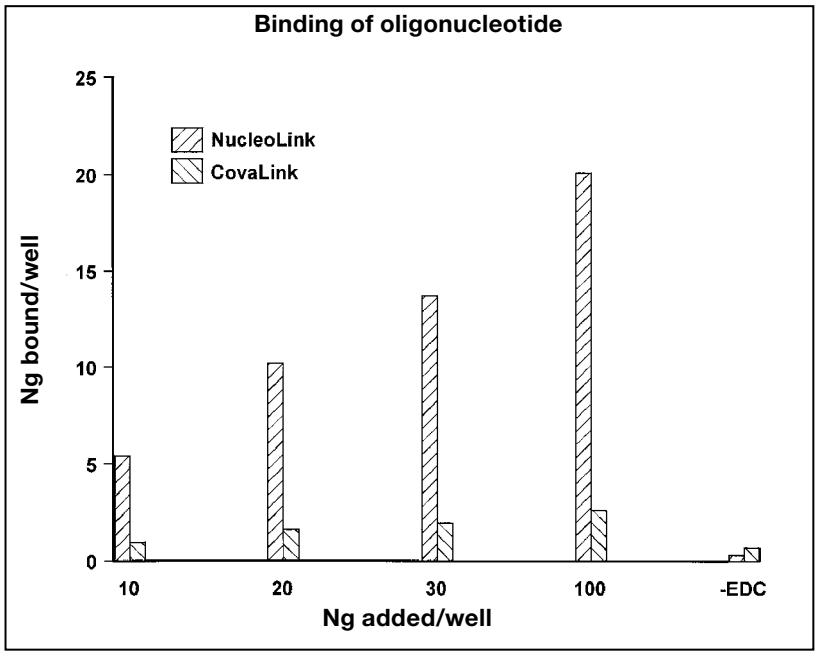


Fig. 1.

The amount of oligonucleotide (10, 20, 30, and 100 ng/well) present during coating.

Non-specific adsorption was tested by adding 100 ng oligonucleotide/well without EDC.

NucleoLink and CovaLink used for DIAPOPS

Six reaction mixtures were prepared. To four of the mixtures 5 µL from four different known positive BLV (Bovine Leukemia virus) samples were added. To the two remaining reaction mixtures 5 µL water were added. One half of each reaction mixture was added to a NucleoLink Strip, the other half to a CovaLink Strip. After amplification, hybridization and incubation with substrate, the fluorescence intensity was measured.

Conclusion

The fluorescence intensities obtained from the positive samples in NucleoLink Strips are approximately 4 times greater than the values from the same samples in CovaLink Strips (Fig. 2). Furthermore, the fluorescence intensities obtained from negative samples in either strips are identical. Therefore, the use of NucleoLink for solid phase PCR assays greatly enhances the distinction between positive and negative samples.

Based on experience, the use of a primer longer than 25 bases for coating is recommended.

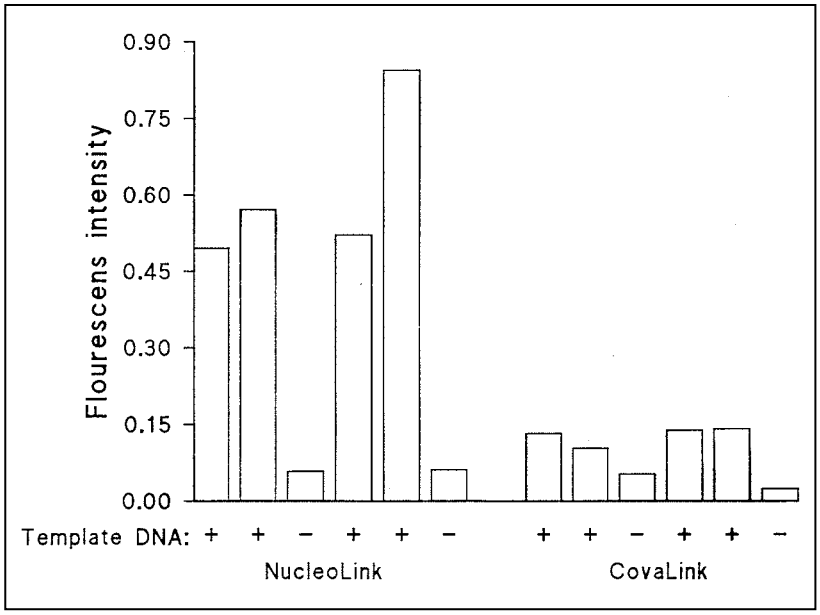
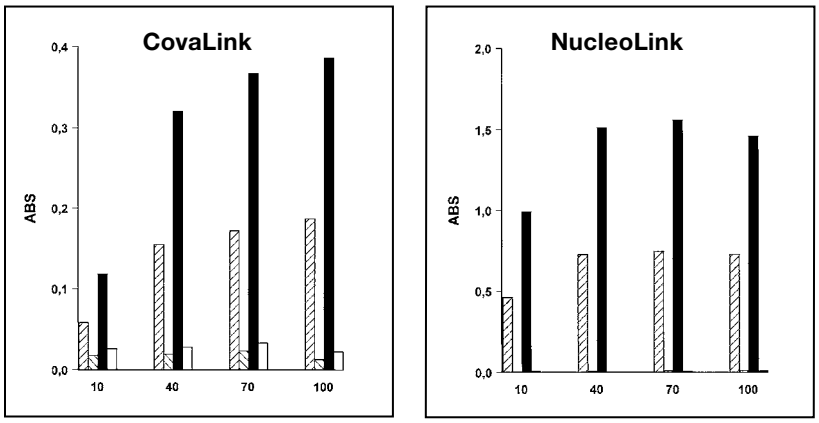


Fig. 2

DIAPOPS ratios obtained in NucleoLink and CovaLink respectively.

+: 5 µL template DNA added before amplification.

−: 5 µL water added before amplification.



Figs. 3 and 4

▨ 1 hour incubation with substrate

▧ background after 1 hour incubation with substrate

■ 2 hours incubation with substrate

□ background after 2 hours incubation with substrate.
Uncoated strips were used for background measurements.

Note the difference in scale on the y-axis.

NucleoLink and CovaLink used for DNA hybridization

Statens Serum Institut (SSI), Copenhagen, has developed a DNA hybridization assay for the detection of infectious organisms. The capture probe was bound as described above to CovaLink and NucleoLink. For the remainder of the assay, the CovaLink and NucleoLink Strips were used in parallel. After hybridization a chromogenic substrate was added. The colour development was measured on an ELISA plate reader.

Conclusion

The results (Figs. 3 and 4) showed that by using NucleoLink, the amount of DNA needed for covalent immobilisation could be reduced by 90%. Furthermore, when using NucleoLink, the time needed for substrate convertion could be reduced by 50%.

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