# Thermo Scientific Nunc NucleoLink Product Guide

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# Contents:

# Product Information

## Format

Which format does NucleoLink have?

NucleoLink consists of thin walled 8-well strips for assembly in a frame, which is of 96 Thermo Scientific MicroWell format.

## Application

What are the main applications of NucleoLink Strips?

The thin walled NucleoLink Strips are optimized for Solid Phase DNA Amplification. By using the NucleoLink Strips conventional and time consuming methods such as Gel Electrophoresis and Southern Blotting are replaced by a much faster "ELISA-like" procedure: DIAPOPS (Detection of Immobilized Amplified Product in a One Phase System), which is a technique that combines Solid Phase PCR and detection by hybridization. The strips can also be used solely for hybridization.

How easy is it to use NucleoLink?

It is very easy, as there is no need to transport the amplicon to a second vessel for detection after amplification. The possibility to use "ELISA-like" procedures (e.g.: ELISA conjugates, substrates and instruments) for the detection as an alternative to radioactivity makes it convenient and safe to use[3].

*The Polymerase Chain Reaction (PCR) process is covered by US patents owned by Hoffmann-La Roche, Inc. and F. Hoffmann-La Roche Ltd.*

# Product Information

## NucleoLink versus CovaLink

What is the main difference between NucleoLink and CovaLink?

When used for nucleic acid (DNA and RNA) assays, the NucleoLink product provides a more heat stable surface than the CovaLink, while maintaining the covalent high binding capacity.

For which applications will NucleoLink substitute CovaLink?

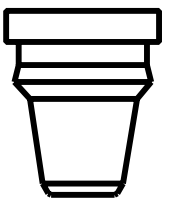
For DNA and RNA applications. CovaLink could be preferred in some hybridization assays where a higher working volume is desired. CovaLink Modules may also be used in some solid phase PCR assays, or other assays requiring a solely 5' coupling of the nucleic acid to the surface.

What are the main advantages of NucleoLink?

NucleoLink Strips are optimized for solid phase DNA amplification (thin walls)[4], stable solid phase up to 120°C and are compatible to a range of thermocyclers[5]. NucleoLink has a much higher binding capacity and saves time and costs[3].

## Features

**What Is the recommended working volume for NucleoLink?**




**Which material is NucleoLink made of?**

An activated heat stable polymer formulated by Nunc.

**How are the strips sealed?**

Tape sealed - using heat stable Tape 8.

**Can the strips be autoclaved?**

When using NucleoLink Strips, it is not necessary to autoclave.

**Which temperatures are the strips resistant to?**

From -20 to 120°C.

## Binding

**Which kind of molecules do the NucleoLink Strips bind?**

Nucleic acids. Primers must be 5' phosphorylated.

**How are the nucleic acids bound to the wells?**

Covalently via carbodiimide condensation.

Is it possible to store oligonucleotides covalently bound to a solid phase?

Oligonucleotides covalently bound to a solid phase can be used after prolonged storage at 4°C.

## DIAPOPS

What is the DIAPOPS technique?

DIAPOPS (Detection of Immobilized Amplified Product in a One Phase System) is a technique where the same well is used for both amplification by solid phase PCR and subsequent detection by hybridization. Manipulation is simplified and contamination diminished since the transfer of amplicon from the amplification system to the detection system is eliminated[1].

What can DIAPOPS be used for?

DIAPOPS can be incorporated into a variety of screening and diagnostic probe assays, utilizing nucleic acid molecules for hybridization, amplification and detection.

Can DIAPOPS be used for quantitative analysis?

Yes. Using the DIAPOPS procedure, a linear relationship between fluorescence signals and template is obtained for more than four logs of template concentrations[3].

| | |
|---|---|
| How long does it take to perform the DIAPOPS assay? | Less than 3 hours with precoated strips (strips with one of the primers bound). |
| What is the detection limit? | 10-100 molecules per well. |

## Detection systems

| | |
|---|---|
| Which kind of detection methods have previously been used? | Colorimetric, fluorescent and radioactive. |

## Instrumentation

| | |
|---|---|
| Which thermocyclers are compatible with NucleoLink™? | Perkin Elmer 9600, MJ Research PTC 200. Techne Gene E[5]. |
| Can standard MicroWell™ plate readers be used? | NucleoLink can be used in MicroWell format readers. |
| Can a multichannel pipette be used? | NucleoLink can be handled by equipment and multichannel pipettes designed for MicroWell format. |

## Reduced risk of contamination

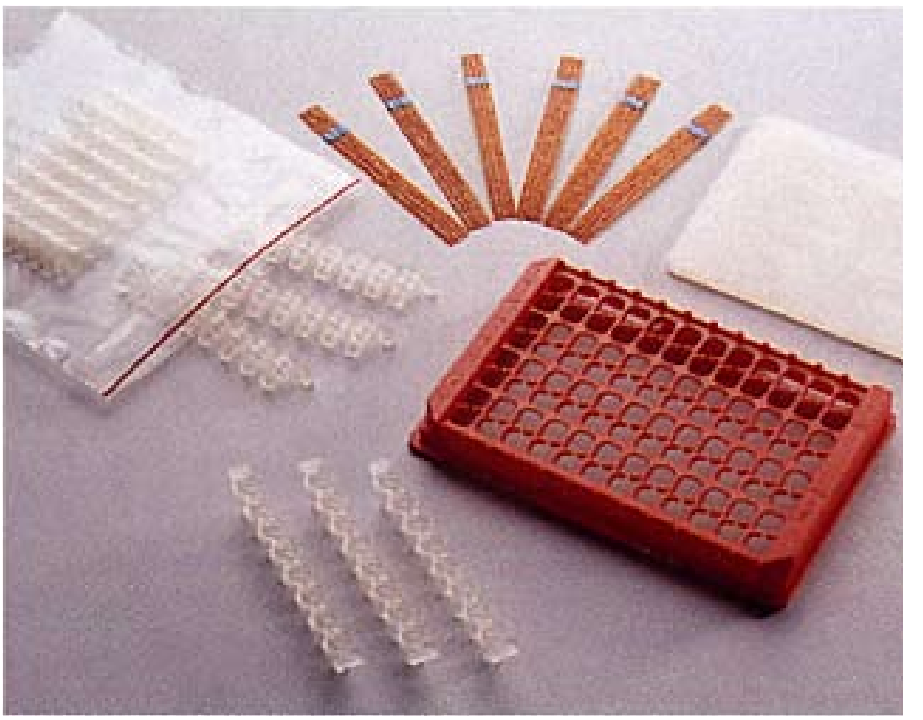
| | |
|---|---|
| Is there a risk of contamination between the wells? | No, negative and positive samples can be run in adjoining wells without cross contamination[5]. |
| Is the contamination risk in the laboratory reduced when using NucleoLink? | Yes, the risk of contamination is diminished since the transfer of amplicon from the amplification system to the detection system is eliminated. |

## Quality Control

| | |
|---|---|
| How is the quality of NucleoLink controlled? | Thermo Fisher Scientific certifies the PCR performance of the NucleoLink Strips. Every sleeve of Strips contains our certificate of performance. The DIAPOPS technique is used as a quality test of the performance of NucleoLink[3]. |

# Handling NucleoLink Strips

using the DIAPOPS technique



**1.**

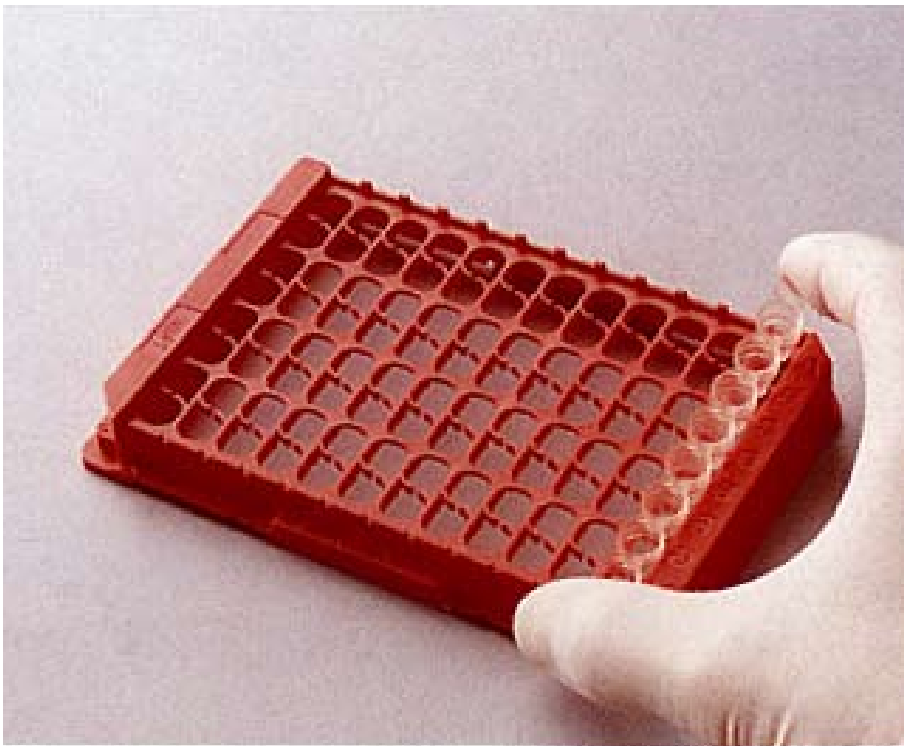
NucleoLink Strips, Tape 8, Spacer plate and Frame.

**2.**

The NucleoLink Strips are packed in a zip bag and can be assembled in the required number.

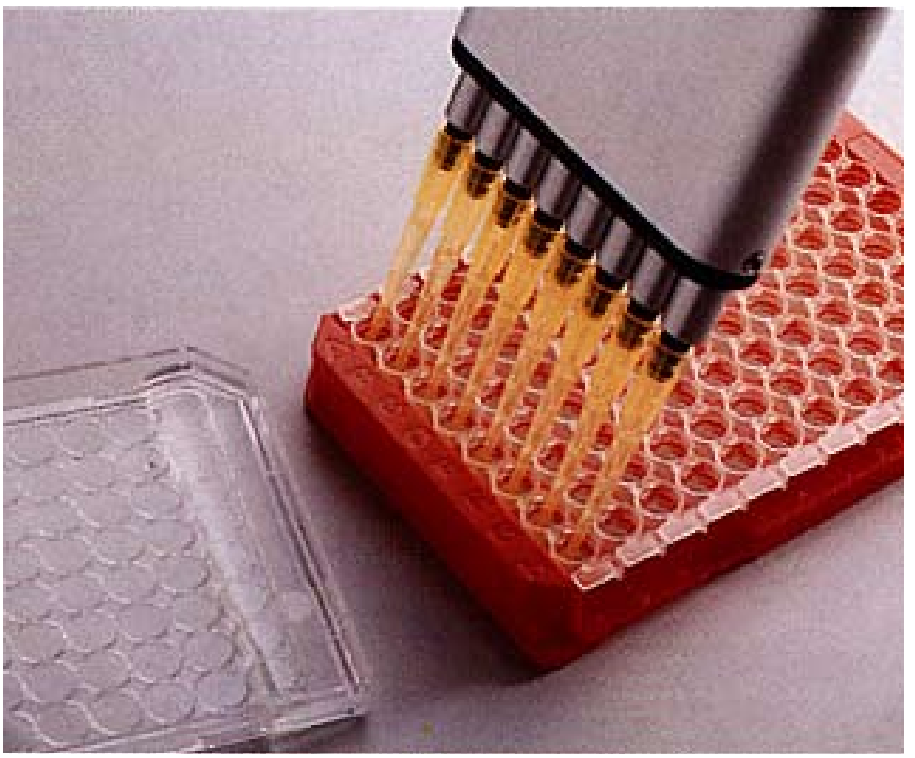
**3.**

The NucleoLink Strips are inserted in a standard MicroWell frame for easy handling.



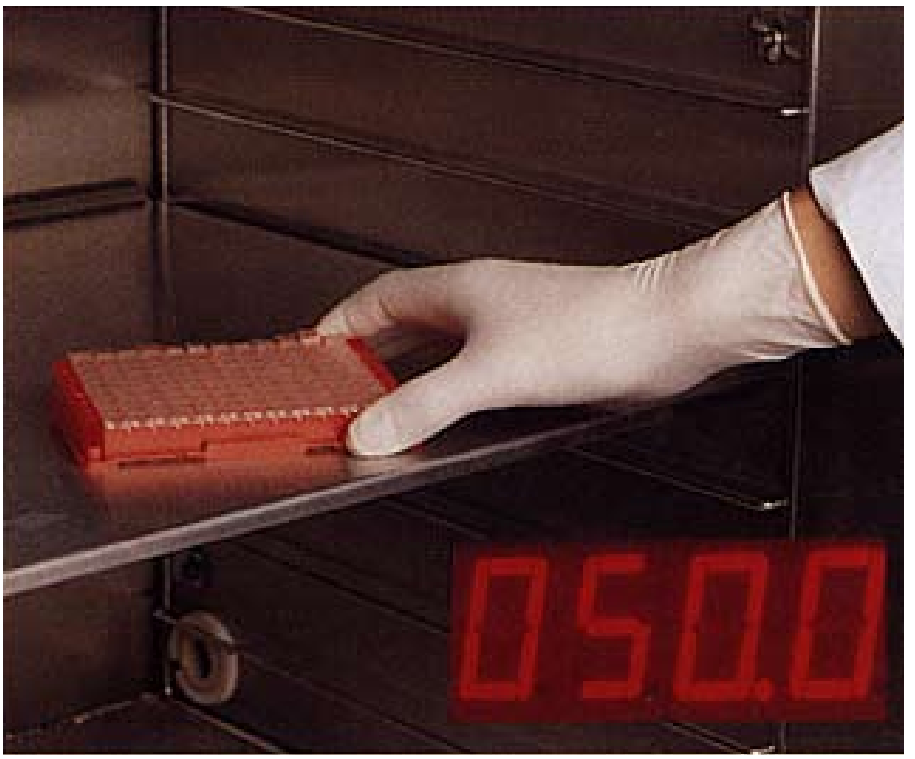
**4.**

Phosphory-lated and carbodiimide solution is added to all wells.



**5.**

The wells are sealed with tape to avoid evaporation.

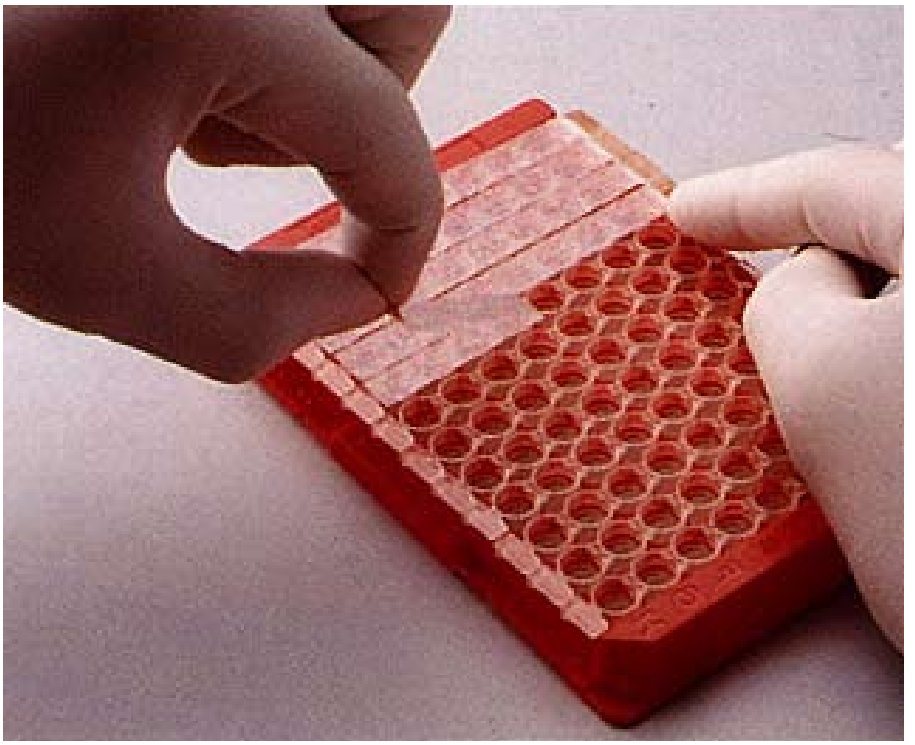



**6.**

The primer is covalently attached to the well during five hours of incubation at 50°C.

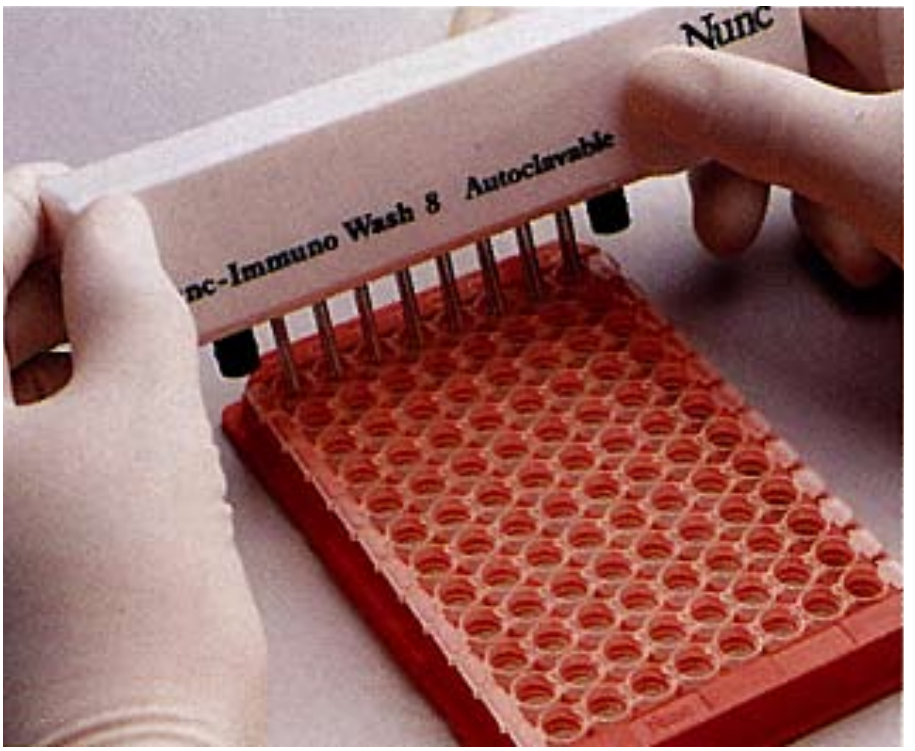
**7.**

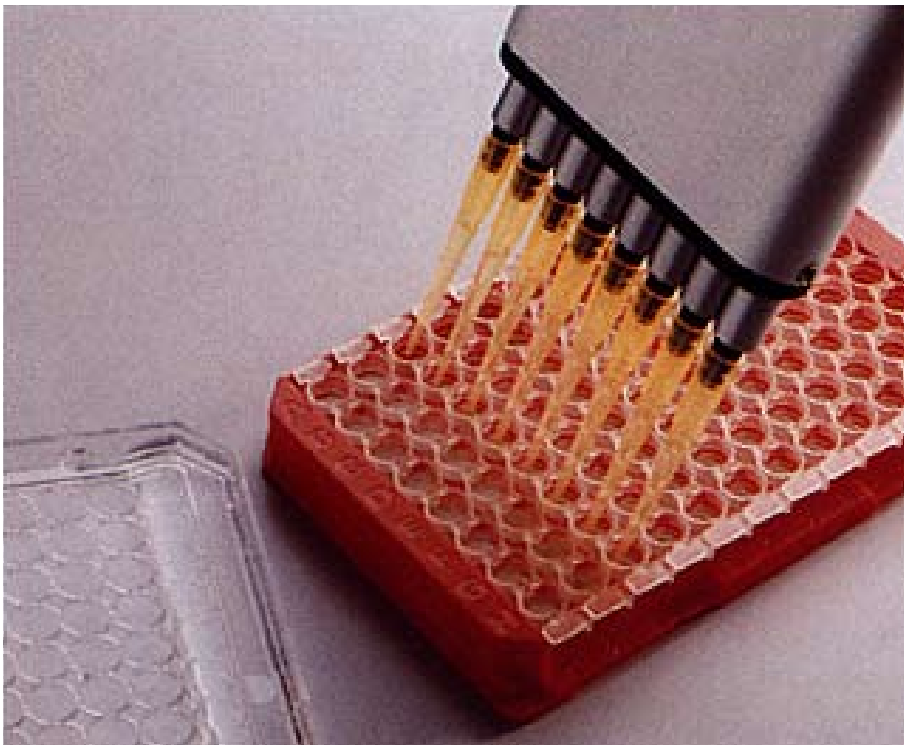
The tape is removed after incubation.



**8.**

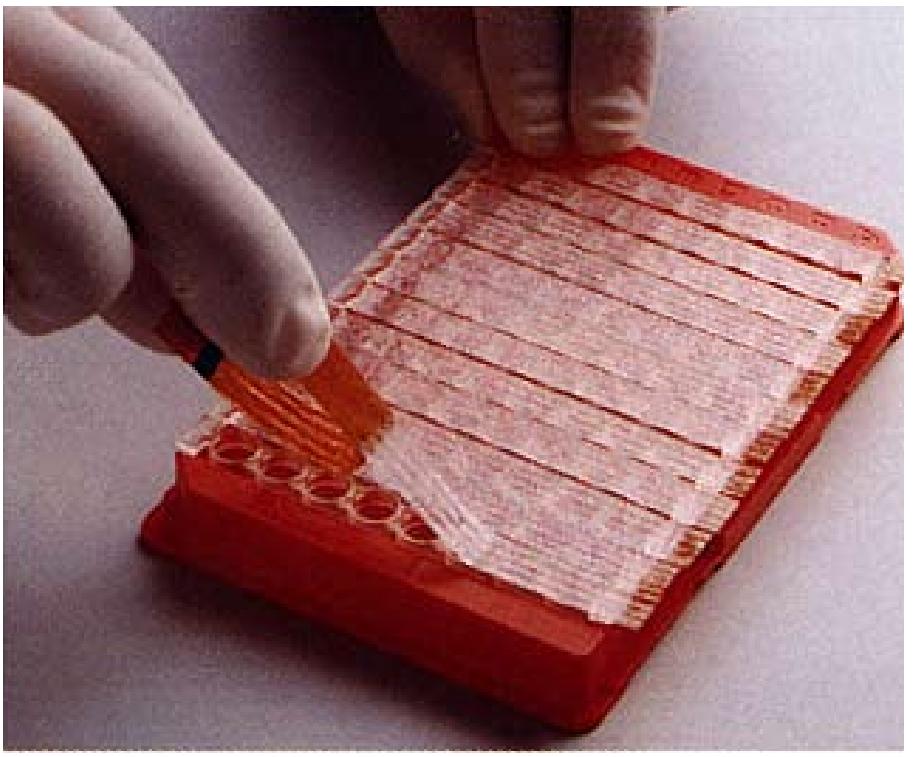
The wells are washed to remove non bound primer.





**9.**

PCR solution (dNTP, primers, Taq, template) is added to all wells.

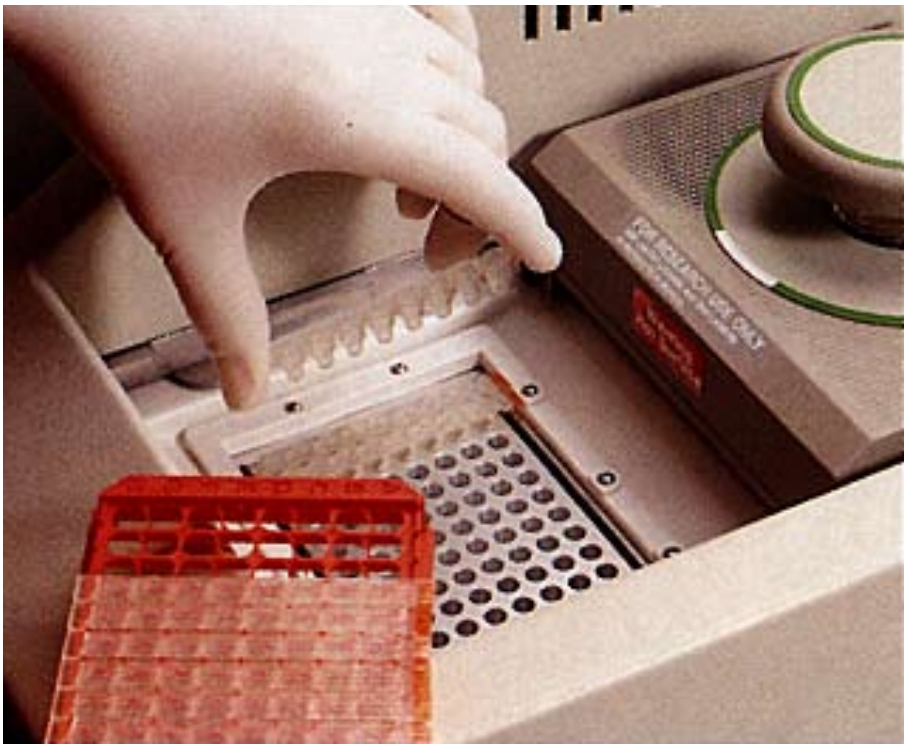


**10.**

The wells are sealed with tape to avoid evaporation during the PCR process.

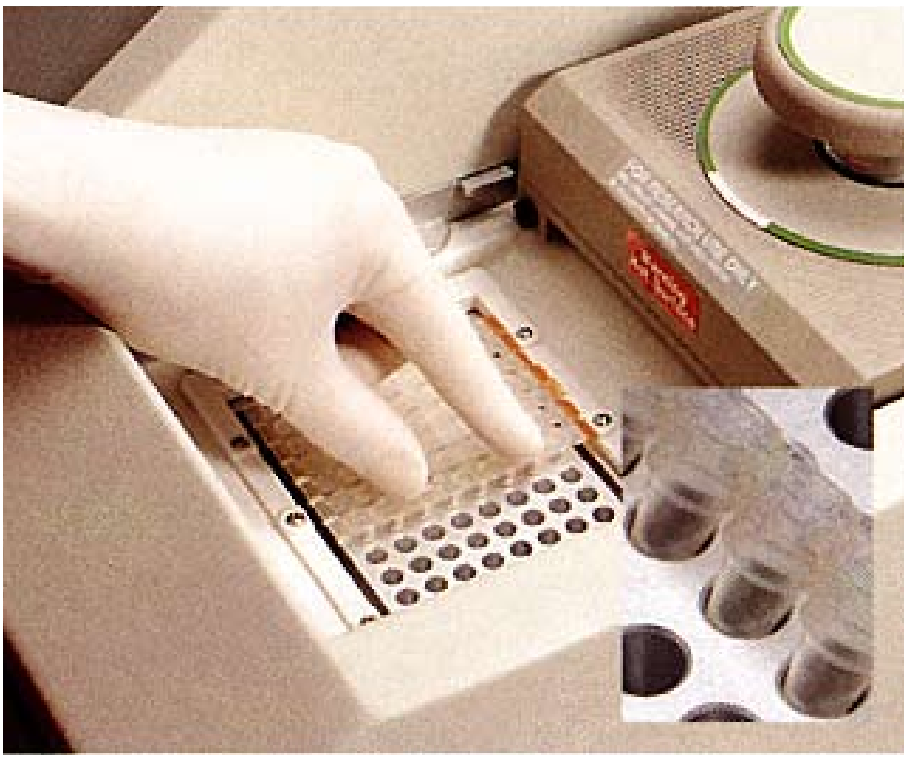
**11.**

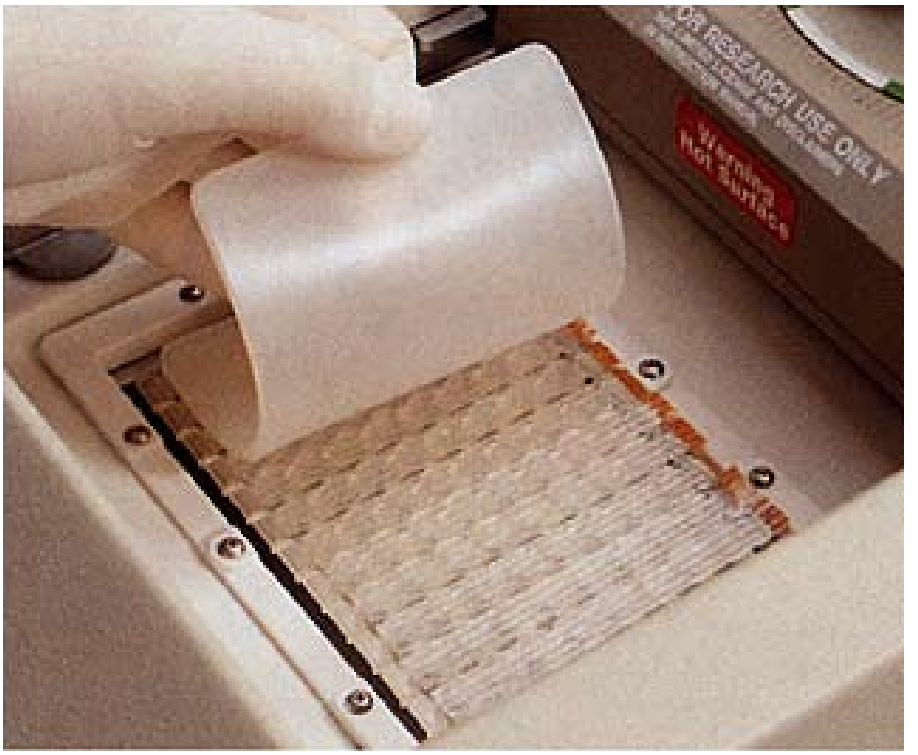
The NucleoLink Strips are mounted in the thermo-cycler.



**12.**

The NucleoLink strips fit perfectly in 0.2 ml blocks.





**13.**

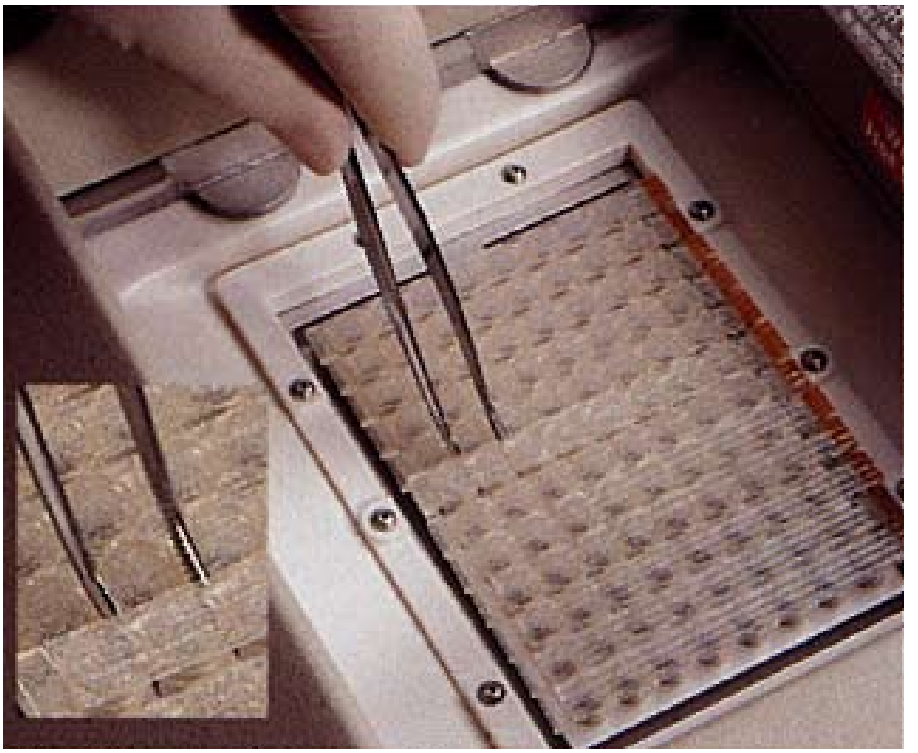
The spacer plate is placed on top of the NucleoLink Strips

**14.**

The lid of the thermo-cycler gives a uniform pressure to all the NucleoLink Strips.

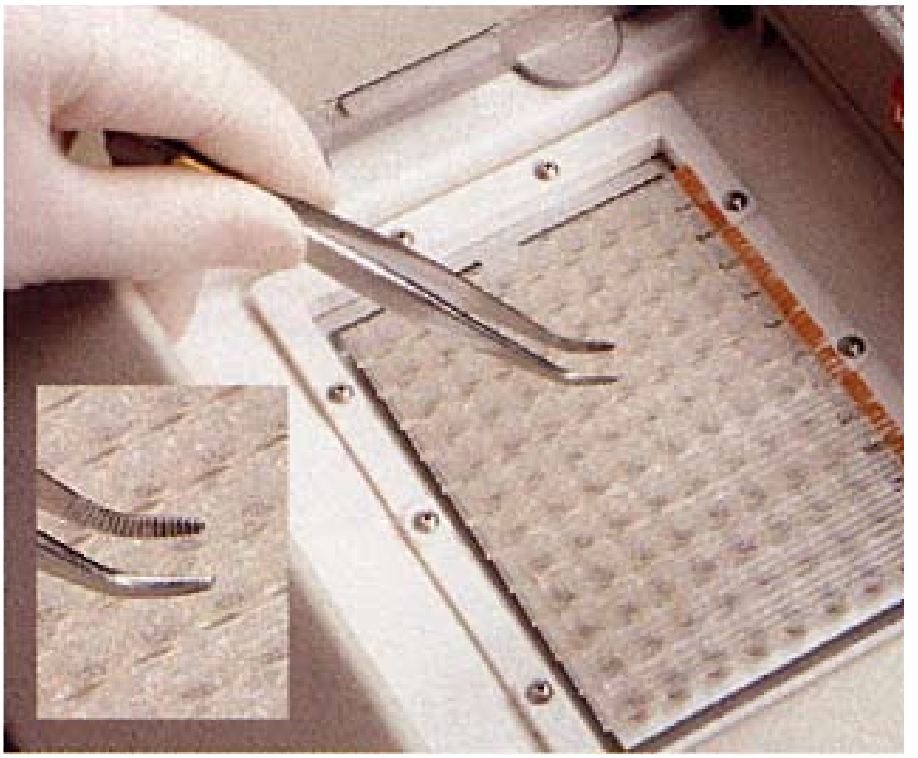
**15.**

The NucleoLink Strips are released from the heating block by using standard angle pincers.



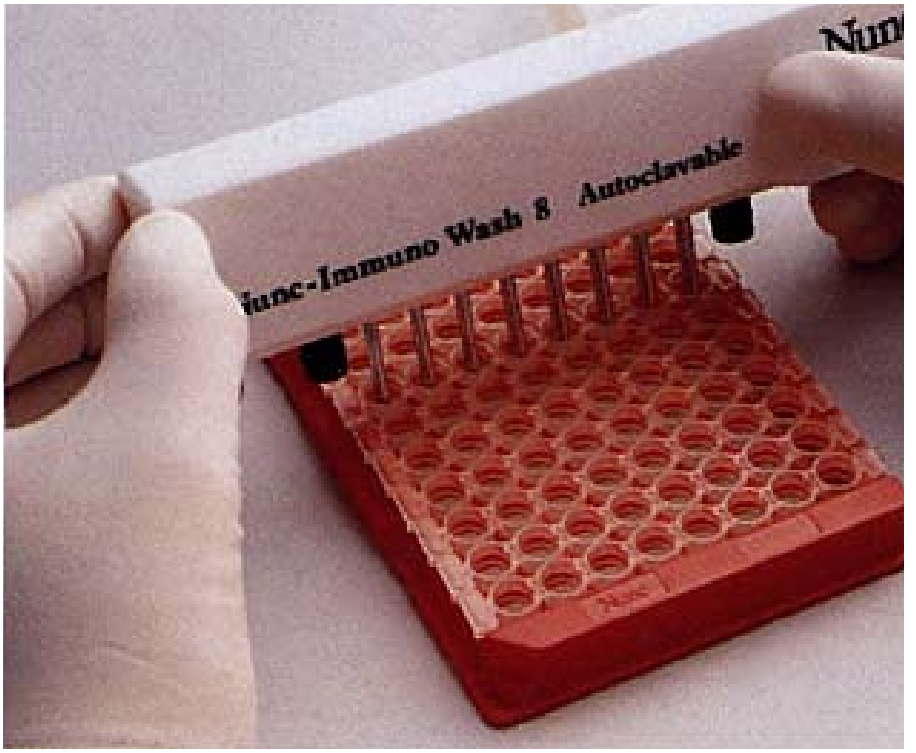
**16.**

View of push out tool (angle pincers).



**17.**

The NucleoLink Strips are removed from the thermo-cycler and mounted in the frame.

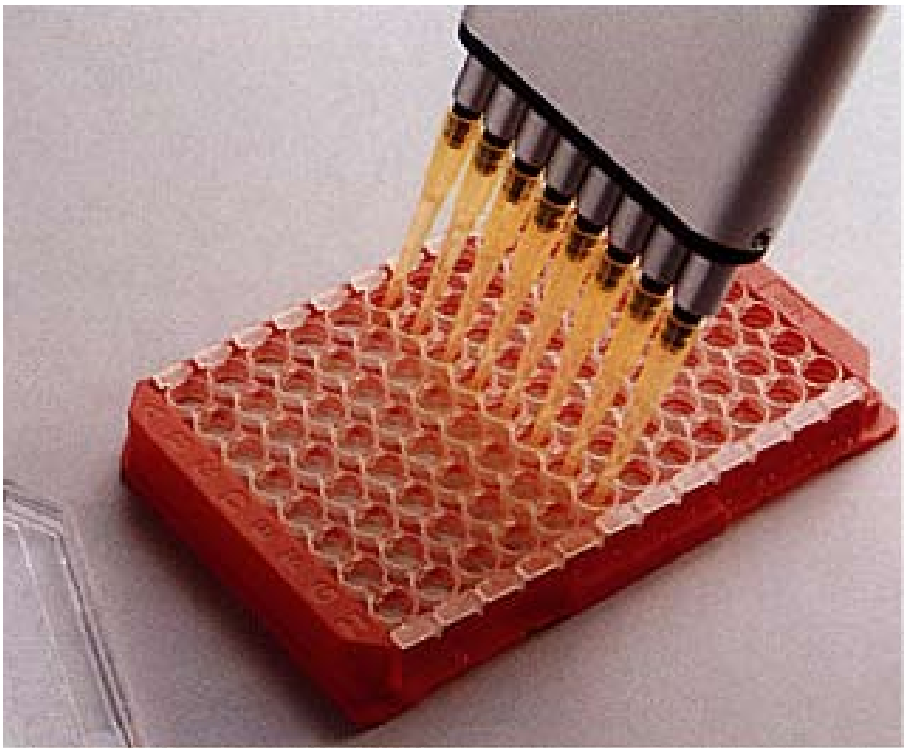



**18.**

Non-covalently immobilized amplicons and PCR reagents are removed by washing.

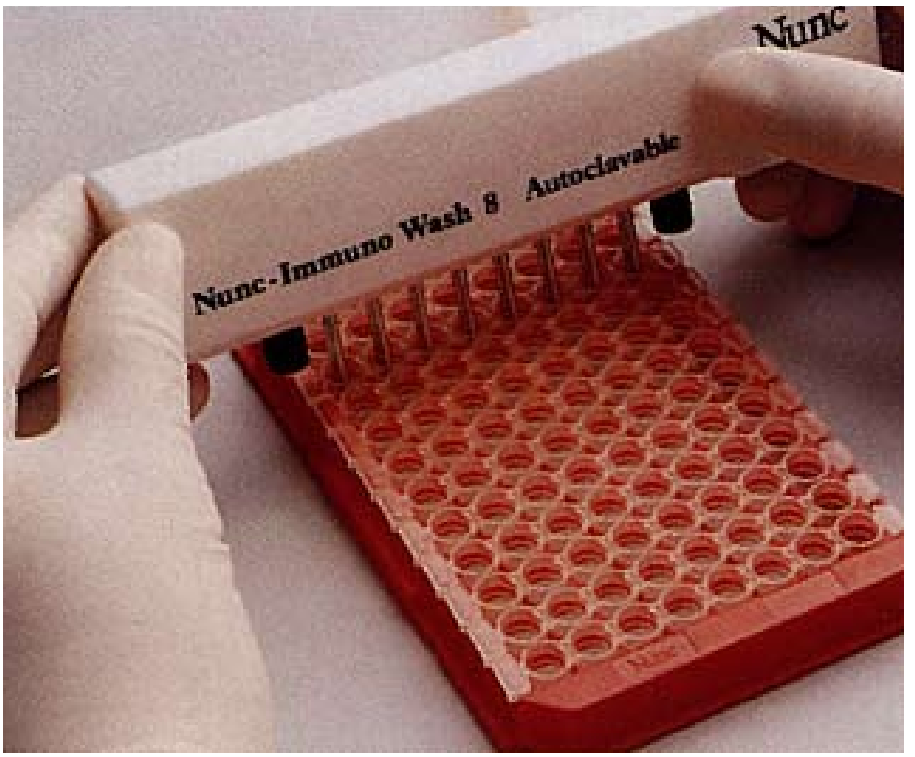
**19.**

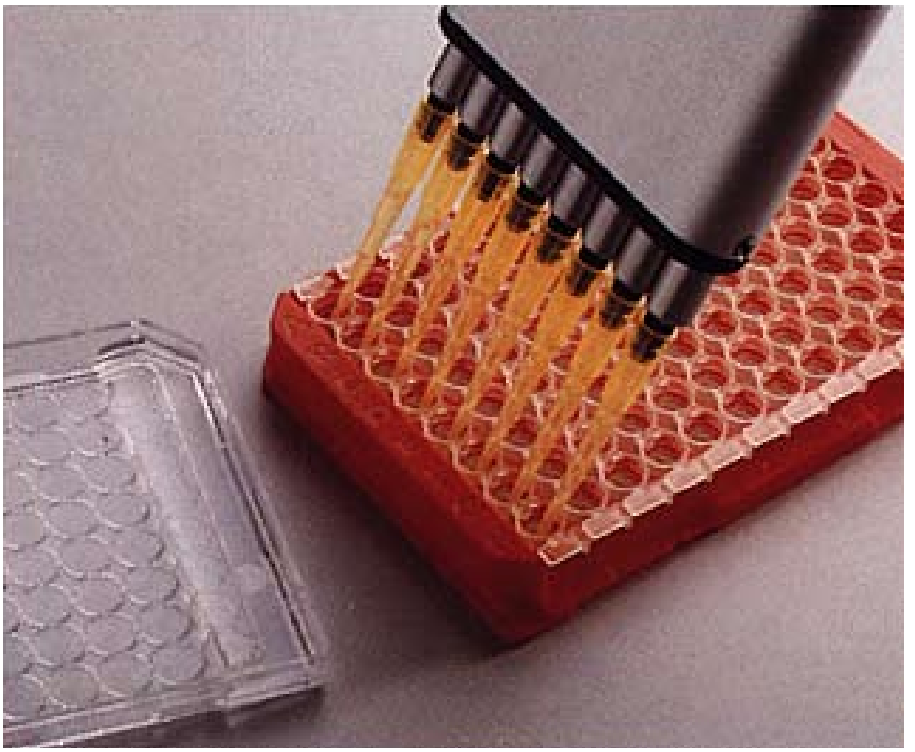
The biotinylated detection probe is added to all wells.



**20.**

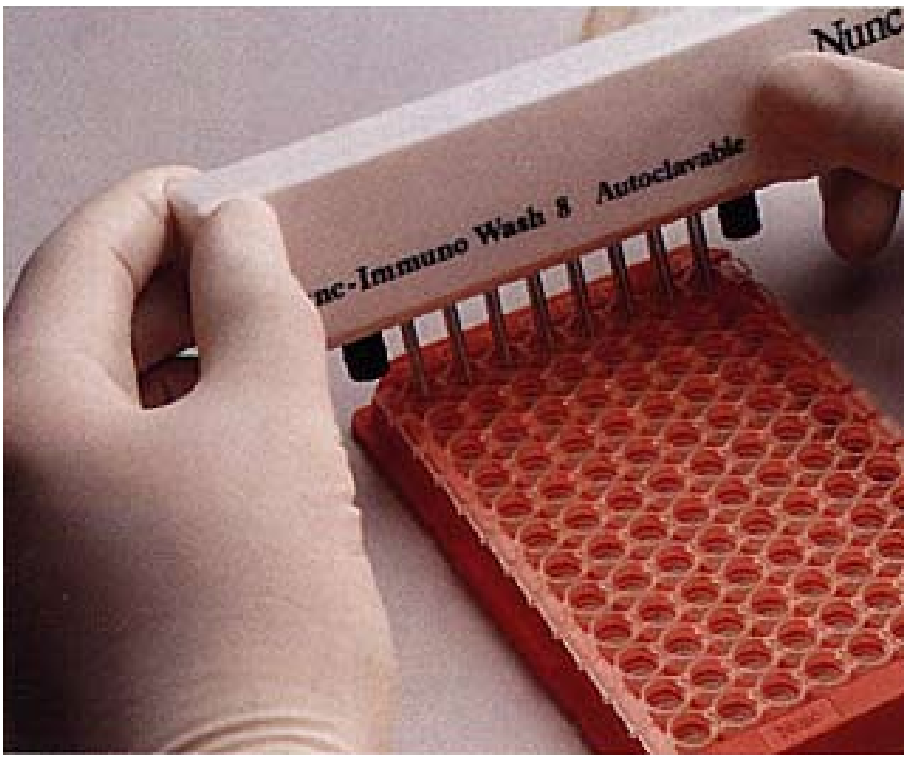
The wells are washed after incubation.





**21.**

Alkaline phosphatase labeled streptavidine is added to all wells.

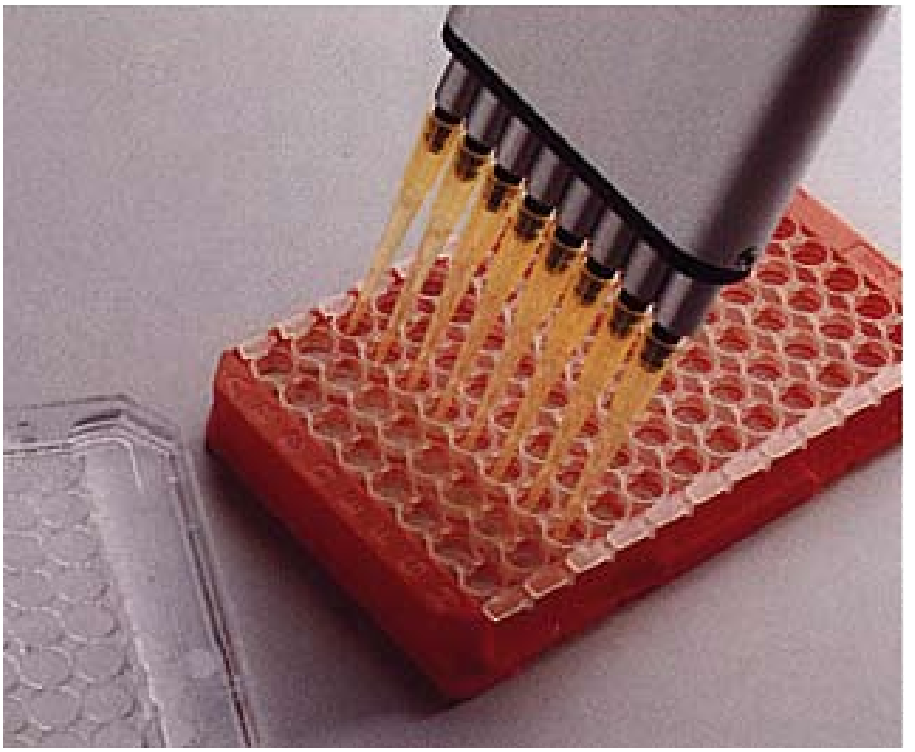



**22.**

The wells are washed after incubation to remove non-bound labeled streptavidine.

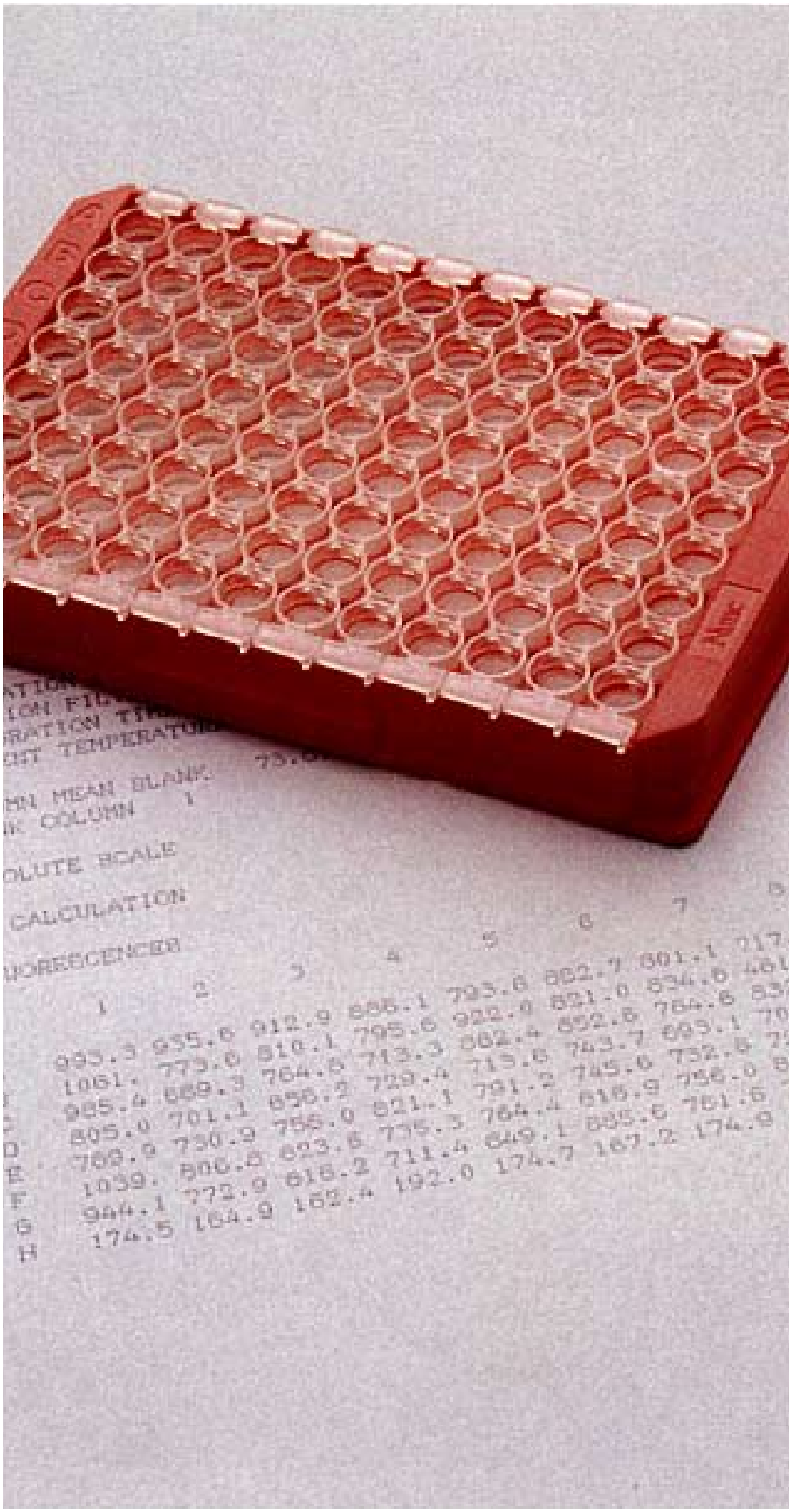
**23.**

Fluorescent substrate (4-MUP) is added to all wells.



**24.**

The wells are read in a fluorometer after incubation.



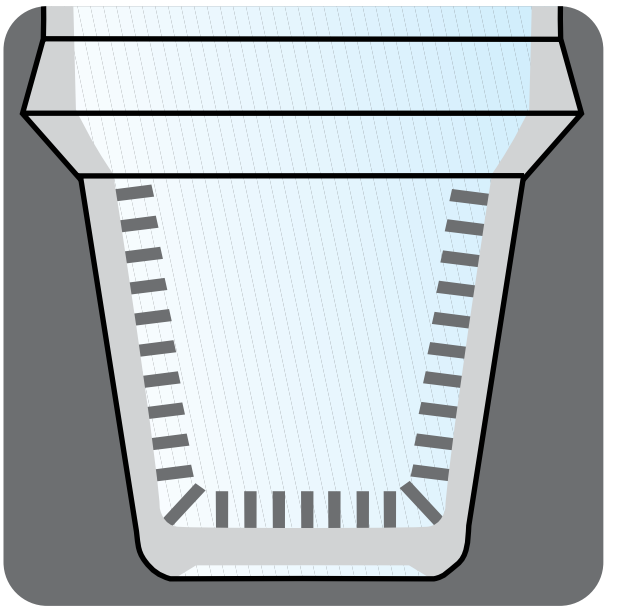
**25.**

Final result and data reduction.

# Principle of DIAPOPS

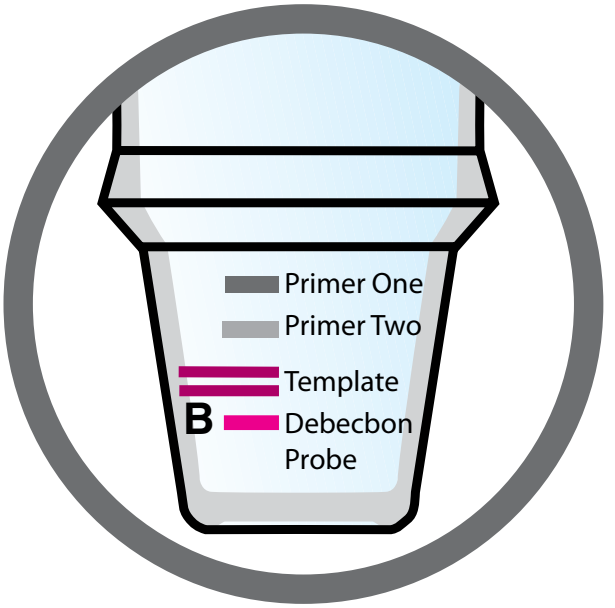
## Binding

Primer One is bound covalently to the surface of a well. For reasons of simplicity we have named the Primers One and Two, but in the actual assay Primer One can be any of the two primers.



## Addition of Reagents

Buffer, Nucleotides. Taq polymerase, Template, Primers One and Two are added to the liquid phase. In the liquid phase the ratio between Primer One and Primer Two should be 1:8.




## Amplification

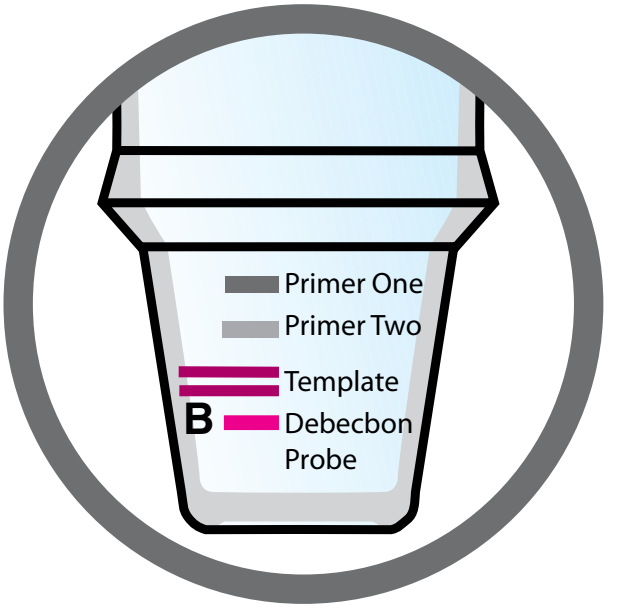
Amplification is initiated in the liquid phase. During amplification, amplicons will hybridize with the bound primer molecules. These primer molecules will be extended by the Taq polymerase.

## Two types of amplicon

After amplification the well contains two types of amplicons: those in the liquid phase and those bound to the well.

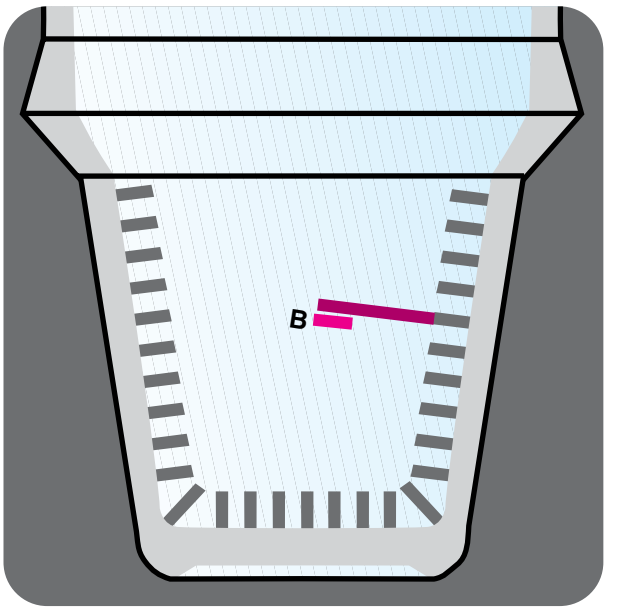
## Denaturation

Amplicon in the liquid phase is removed by washing. The bound amplicon is converted to single stranded molecules (denatured) by treatment with NaOH.




## Detection

The single-stranded molecules can be detected by hybridization with any detection probe. When "ELISA-like" procedures are used for detection, the result can be read in an ELISA or a fluorescence reader.



# NucleoLink Product Overview

| Cat. No. | Description | Units per Sleeve/Carton/Case | Material |
|---|---|---|---|
| 248259 ‡ | NucleoLink Strips. Clear | 12/120/1440 | Activated heat stable Polymer |
| 249182 ‡ | Frame | 6/72 | Acrylonitrile-Buta-diene-Styrene (ABS) |
| 250105 ‡ | Tape 8 and Spacer Plate | Tape: 60/480/5760<br>Spacer: 1/12 | Polyester<br>(adhesive: Silicone) |
| 248150 ‡ | 96 well plate with C well | 5/30 | Activated heat-stable polymer |
| 248450 ‡ | Break Apart Module with 8 wells | 5/30 | Activated heat-stable polymer |
| 248262 ‡ | Strip with 8 wells. White | 12/120/1440 | Activated heat-stable polymer |
| 248650 ‡ | Strip with 8 wells. Black | 12/120/1440 | Activated heat-stable polymer |
| 232702 | Amplification Tape 96 | 100/100 | Polyolefin<br>(adhesive: Pressure sensitive acrylate) |
| 259684* | Spacer Plate | 1/1 | Silicone |
| 249344 ‡ | NucleoLink Starter Kit | 1/12<br>(48 NucleoLink strips, 1 Frame, 60 Tape 8,1 Spacer Plate and Application Literature/kit) | |

*‡ Not available in Americas*

** Reusable and autoclavable*

# References


1. Rasmussen, Soren et al, "Combined Polymerase Chain Reaction-Hybridization Microplate assay used to Detect Bovine Leukemia Virus and Salmonella". Clin. Chem. 40/2,200-205 (1994).

2. Chevrier, Danièle et al, "PCR product quantification by non-radioactive hybridization procedures using an oligonucleotide covalently bound to microwells", Mol. and Cell. Probes (1993) 7,187-197.

3. Nunc TechNote Vol 3 No.17 NucleoLink versus CovaLink Surfaces.

4. Nunc TechNote Vol 3 No.18 Thermal profiles of liquid in NucleoLink and Top Yield Strips and Perkin Elmer thin wall tubes in the Perkin Elmer 9600 Thermal cycler.

5. Nunc TechNote Vol 3 No.19 NucleoLink and Top Yield Strips as traditional amplification tubes in commercial thermal cyclers.

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