

**nunc™**



**NUNC MICROARRAY SLIDES**

Glass  
**Oligo Binding**

**INSTRUCTION MANUAL**

Version 1.0

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## Introduction

Using its expertise in surface chemistry Nunc has developed a modified polymer surface for glass slides ideal for oligo-nucleotide microarray printing. The polymer surface is intended for covalently binding aminomodified oligonucleotides 10-100 mer in length.

The glass substrate is made using etching and softplama deposition creating an exceptionally uniform surface.

The hydrophobic surface ensures clear, compact printed spots.

The activated surface is produced under controlled conditions to ensure slides with a dust free and uniform surface. All batches are QC tested for uniformity and functionality.

Nunc Oligo slides are all barcoded facilitating orientation, handling and ensuring 100% traceability.

**Note:**

*Only the side with the affixed label has been treated for immobilization.*

## 1. Handling and Care

Please follow these guidelines to ensure optimal performance:

Nunc Oligo slides have been carefully processed and packed in slide mailers. Avoid direct contact with the printing area, as this may influence binding and lead to a high background fluorescent signal.

Use the slides in a clean environment. Particulate contaminants on the slide surface may jeopardize the array being printed or the scanned signal.

The surface is pH stable within the range pH 3 – pH 11.

The surface tolerates boiling.

Please note: Due to signal intensity achieved with a laser scanner, it is advisable to modulate the scanning image by decreasing the PMT and/or the laser power.

Powder free latex gloves are recommended when handling Nunc Oligo MicroArray Slides

Coloured inks of permanent markers may interfere with fluorescent images. If you use air-spray for drying or cleaning slides before scanning- be sure to use sprays that do not contain fluorescent propellant.

**Note:**

*Spotting buffers containing DMSO, Tween 20 or other detergents are not recommended. Even trace amounts of Tween 20 will inhibit the covalent coupling.*

We recommend opened packages of Nunc Slides be stored desiccated at room temperature (20 - 25°C) until use.

## 2. Immobilisation of Oligonucleotide to Nunc Oligo MicroArray Slides

Commonly used procedures for printing and hybridisation (e.g. hybridisation including formamide) will provide good results with Nunc Oligo MicroArray slides.

NUNC recommends the protocol below.

### 2 A. Binding of Oligonucleotides

An efficient binding to the slides is obtained when the oligonucleotides are amino-modified on the 5'- or 3'-terminal end.

### 2 B. Prior to Spotting

The optimal printing buffers for micro spotting are 1 x Borate buffer; pH 10 or 1 x Betaine-Citrate buffer; pH 7 with a recommended spotting concentration of oligonucleotides ranging from 10 – 50  $\mu$ M. Due to the hygroscopic effect of betaine, the Betaine-Citrate buffer is recommended when many slides are printed. (For the preparation of printing buffers, see 4. Protocols Preparation of Buffers etc.).

**Note:**

*DNA-molecules to be spotted are not to be prepared in solutions with aliphatic amino groups (e.g. Tris buffer), detergents (e.g. TWEEN 20, SDS) or nucleophiles (e.g. ethanolamine, lysine and free ammonium), as they will inhibit the covalent coupling.*

### 2 C. Printing and Immobilisation

- 1) Print the DNA samples on the activated side of the slides.
- 2) Place slides in a humidity chamber (75% relative humidity) with saturated sodium chloride at room temperature for overnight incubation.  
(How to make humid chamber, see Protocols p. 7.)

## 2 D. Blocking and Washing

- 1) After immobilisation, residual reactive groups are blocked for 1 minute by agitation in Washing Solution I. (For the preparation of Washing Solution I, see Protocols p. 7.) Use approx. 40 ml washing solution for each slide. Repeat this step 3 times.
- 2) Soak the slides in Washing Solution I for 5 minutes at room temperature.
- 3) Discard Washing Solution I and rinse the slides for 5 minutes by agitation in distilled water at room temperature.
- 4) Dry the slides by centrifugation (800 x g for 3 minutes) or blow dry using compressed N<sub>2</sub>.
- 5) Store the slides desiccated until use.

## 3. Hybridisation to Immobilised Oligonucleotide Arrays

### 3 A. Hybridisation

- 1) Prepare the labelled and purified hybridisation probe in Hybridisation Buffer and denature the mixture for 3 minutes at 95°C and pulse a few seconds in a microcentrifuge. (For the preparation of Hybridisation Buffer, see Protocols p. 7).
- 2) Add the denatured hybridisation mixture to the array and cover liquid with a Nunc LifterSlip. Make sure the hybridisation mixture cover the printed area completely, without bubbles and without significant waste due to overflow.
- 3) Incubate slides horizontally overnight in 100 % humidity at the appropriate hybridisation temperature.

**Note:**

*When using fluorescent probes, perform hybridisation in a dark environment and generally avoid exposure to light.*

### 3 B. Washing

To eliminate background noise, use fresh wash baths for each run and make sure the slides do not dry out between the washing steps. If several slides have been hybridised with different probes they should not be washed in the same washing container.

- 1) Carefully remove coverslips from the slides by soaking in 4 x SSC.
- 2) Wash slides for 1 minute with agitation in 4 x SSC; 0.005 % Tween 20, at room temperature.
- 3) Wash slides for 1 minute with agitation in 0.1 x SSC; 0.005 % Tween 20, at room temperature.
- 4) Dry slide by centrifugation (800 x g for 3minutes) or blow dry using compressed N<sub>2</sub>. If the slide is air dried, a fluorescent haze may occur, therefore blow drying or centrifuging off the liquids results in a lower fluorescent background.
- 5) Scan the slides.

## 4. Protocols

### 4 A. Preparation of Buffers etc.

REAGENT	CONTENT	NOTE
2 x Borate Buffer	100 mM Boric Acid, pH 10	Adjust pH to 10 with 1M NaOH.
2 x Betaine-Citrate Buffer	3 M Betaine; 6 x SSC	Use Betaine: Sigma catalog # B 2754
Washing Solution I	150 mM NaCl; 0.1% Tween 20; 100 mM Tris, pH 7.5	Adjust pH to 7.5 with 1 M HCl.
Hybridization Buffer	5 x SSC; 0.1 % SDS; 0.1 µg/µl Salmon Sperm DNA	
10% SDS	10 g Dodecyl Sulfate Sodium salt in 100 ml water.	Dissolve at room temperature.
20 x SSC	3 M NaCl and 0.3 M Sodium Citrate, pH 7.0.	Adjust pH to 7.0 with 1 M HCl.
Humidity chamber		Add as much solid Sodium Chloride to water as needed to form a 1 cm deep slurry in the bottom of a plastic container with airtight lid. This forms a chamber with a relative humidity of approx. 75

## Nunc Products for MicroArray Applications

Cat. No.	Product
230313	MicroArray Slides, Glass, Oligo Binding
230311	MicroArray Slides, Glass, Protein Binding
230379	MicroArray Slides, Black Polymer, Aminosilane
230389	MicroArray Slides, Clear polymer, Aminosilane
230381	MicroArray Slide, Black Polymer, NucleoLink™
230351	MicroArray Slide, Clear Polymer NucleoLink™
230302	MicroArray Slide, Clear Polymer, MaxiSorp™
230305	MicroArray Slide, Black Polymer, MaxiSorp™
375357	Cryotubes 1.0ml
442587	Microwell Plate™ V96, Polypropolene
265196	384 Well Plates, clear
166508	NunclonΔ Dish, 500 cm <sup>2</sup>
168281	NunclonΔ Dish, 145 cm <sup>2</sup>
150350	NunclonΔ Dish, 56.7 cm <sup>2</sup>
373660	Centrifuge Tubes, 50 ml
366036	Centrifuge Tubes, 15 ml
250865	GeNunc™ Tubes, 0.2 ml
270971	GeNunc™ Tubes, 0.5 ml
330050	Scanner for USP

For technical support please contact [microarray@nunc.dk](mailto:microarray@nunc.dk)