

Macro and Micro-Array

Slides



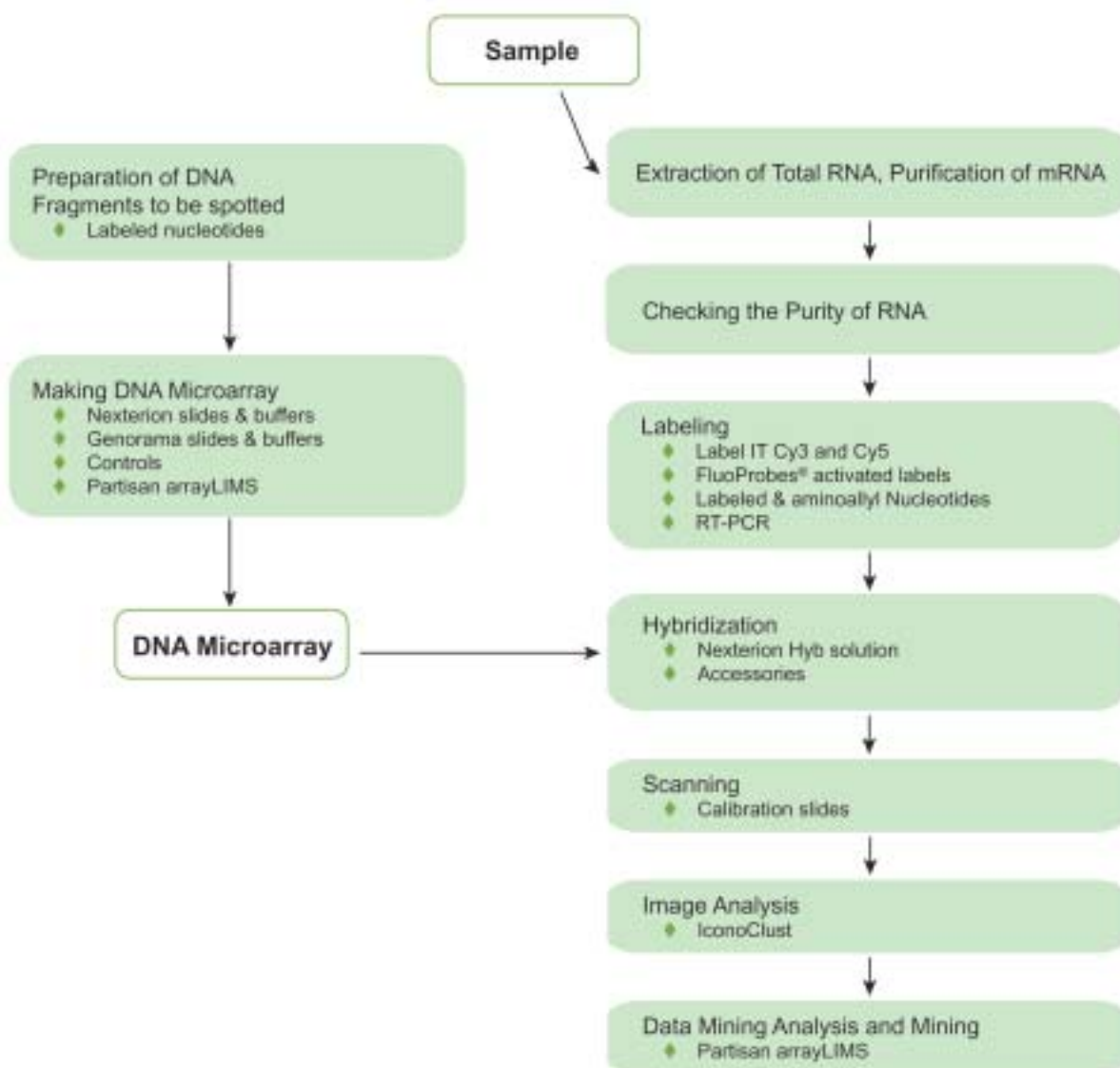
FluoProbes® is pleased to provide state of art reagents for genetics using fluorescence technology. Fluorescence catalyzed developments of many molecular biology techniques including cytogenetic, DNA amplifications and MicroArrays.

MicroArraying

Many biochemical and molecular biological procedures use specific interactions for the detection of individual molecules, like the nucleic acids sequence-specific hybridization. Based on this principle, nucleic acid-microarrays allow the rapid and simultaneous analysis of thousands of genes for expression profiling, mapping, sequencing and polymorphism detection.

This high-throughput method of nucleic acid analysis has defined the field of functional genomics. RNA samples from any cell or tissue type can be analyzed for changes in transcript levels that might indicate their involvement in development, cancer, infection, or response to drug or any other biological process. Microarray technology can also be used for sequencing, genotyping and comparative genomics applications.

For a good coverage of spotted microarray technology, you can read "A molecular cloning manual : DNA Microarray", edited by D. Bowtell & J. Sambrook, 2002, CSHL press. In addition to expression profiling, covered areas include uses of microarrays for analysis of chromatin immunoprecipitation samples, DNA copy number determination, and detection of genetic polymorphism (oligo arrays). The bioinformatic section is less extensive than the experimental parts, but the chapters on clustering, self-organizing maps and databases serve as good introductions to these areas.



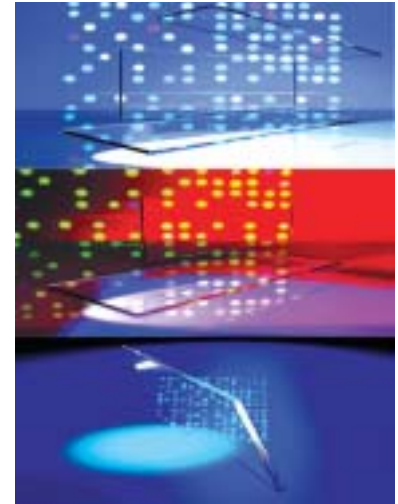
Nexterion™ Microarraying

Microarrays are constructed in a way that probe molecules have predefined positions (spots) within the array. Fluorescence labeled target molecules hybridize to the immobilized probe molecules. Subsequently, microarrays are analyzed e.g. by laser-induced fluorescence measurement.

Nexterion™ Microarray Slides

For **Nexterion™ Slides**, only high quality laser-cut and extremely low-fluorescent glass substrates are used. The special cleaning and chemical coating procedures favor the generation of high-quality microarrays. The dimensions of all Nexterion Slides are 75.6 mm x 25.0 mm x 1.0 mm.

Nexterion Slides can be used for all spotting systems - contact and contact free printing systems. In combination with our optimized Nexterion Solutions and Buffers, the production of DNA microarrays is a quick and easy task.



Application fields of Nexterion™ Slides :

- ◆ Gene expression monitoring of known and unknown genes
- ◆ Mutation detection and analysis
- ◆ Genotyping of eukaryotes, microorganisms, viruses etc.
- ◆ Genes and clones Mapping

	Short oligonucleotides		Long oligonucleotides		PCR-Products		Proteins
	with NH ₂ -Link	without NH ₂ -Link	with NH ₂ -Link	without NH ₂ -Link	with NH ₂ -Link	without NH ₂ -Link	
Nexterion Slide E	+++	++	+++	+++	++	++	+
Nexterion Slide AL	++	-	++	-	+++	++	++
Nexterion Slide A+	+	+	+	++	+	+++	-
Nexterion Slide H*	++	-	+	-	+	-	+++

- : Not recommended

Advantages :

- ◆ Homogeneous coating
- ◆ Optimal chemistry of functional groups
- ◆ Low background fluorescence
- ◆ No batch-to-batch variation
- ◆ Chemically inert barcode labeling

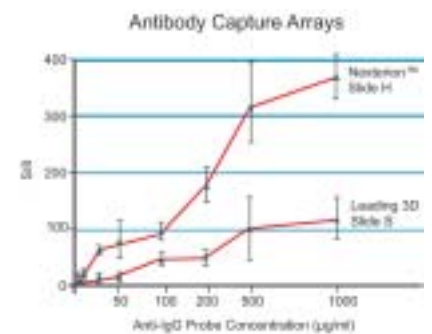
The chemically reactive and homogeneous spotting area is shown for all types of Nexterion Slides.

Nexterion™ Slides are based on high quality borosilicate glass of excellent flatness and low autofluorescence :

Dimensions : 25 x 75.5 mm +/- 0.2 mm

Thickness : 1.0 mm +/- 0.1 mm

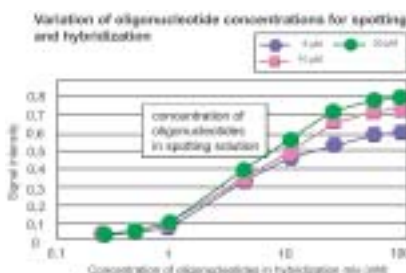
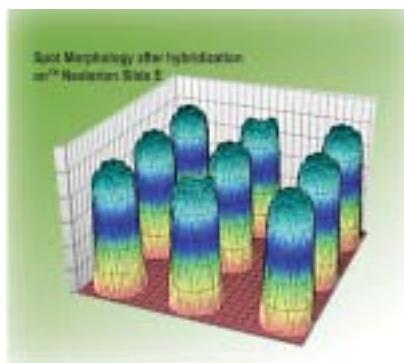
Flatness : +/- 50 µm



Signal-to-background comparison Nexterion® Slide H and major aminosilane competitor slide Human oligonucleotides was hybridized with Cy5™-labeled Total Universal Human RNA and Cy3™-labeled Total Universal Mouse RNA. In courtesy of Dr. Pascal Soularue, CEA, Evry, France.

Macro and Micro-Array

Slides



The graph shows the normalized signal intensities after hybridization with different labeled oligonucleotides concentrations. Oligonucleotides in Nexterion Spot have been spotted at different concentrations onto Slide E.

*reference :
Gunnar Wrobel et al. , 2003 - Nucleic Acids Research, 2003, Vol. 31, No. 12 e67
Optimization of high-density cDNA-microarray protocols by "design of experiments".



Nexterion™ Slide E

Epoxy Slides are especially suitable for covalent immobilization of amine-modified short oligos (10-25 mers), non-amine and amine-modified long oligonucleotides (>25 mers). Nexterion Slide E can also be used for printing cDNA PCR-products. Additional amino-modifications of the nucleic acids are not required. The hydrophobic surface allows small spot diameters (100 to 130 µm) to create high-density arrays. The surface chemistry is very stable and remains active even during very long spotting runs.

Furthermore, also peptides, proteins (for instance antibodies), cells and tissues can be immobilized on Nexterion Slide E. In principle all nucleophilic groups (NH₂-, SH-, OH-) will react immediately and irreversibly with the epoxy group. Additional steps for immobilization like UV-crosslinking are not required.

The recommended final concentration of nucleic acids in spotting buffers is 10 to 20 µM for oligonucleotides and 0.1 to 0.5 µM for PCR-products.

Description	Cat.#	Qty
Nexterion™ Slide E, Starter Kit	T50381	1 kit
Includes : 10 Nexterion Slide E, 10 ml Nexterion Spot, 100 ml Nexterion Block E, 10 ml Nexterion Hyb		
Nexterion™ Slide E, 75.6 mm x 25 mm	U91823	25 each
Nexterion™ Slide E, 75.6 mm x 25 mm bar-coded	U91832	25 each

For the spotting and binding of DNA-probes onto Nexterion Slide E we recommend the optimized spotting buffer Nexterion™ Spot (#T78512). As an alternative, 3x SSC can be used, or 1.5 M betaine in 3x SSC if one want to avoid evaporation of the spotting process. Also, of the combinations surveyed by Gunnar Wrobel *et al.* (2003), combining Nexterion Slide E and FBNC buffer (0.5 M betaine, 25 % (v/v) formamide and 0.5 µg/µl nitrocellulose) as the spotting buffer seems to be the best compromise in terms of spot diameter, signal intensity and sensitivity. This configuration allowed a density of up to 70 000 spots per slide.

Blocking is recommended with Nexterion block E solution (#U76353), and hybridization with Nexterion Hyb solution (#U65433).

NEW ! Nexterion® Slide MPX

- ◆ Parallele analysis of multiple biological samples against focused subsets of probes
- ◆ Excellent reproducibility compared to conventional single-array applications
- ◆ Increased sensitivity and reduced use of limited sample material
- ◆ Substantial reductions in costs per experiment
- ◆ Available in different well formats with all standard functional coating chemistries
- ◆ Compatible with standard commercial printing, liquid handling and scanning equipment

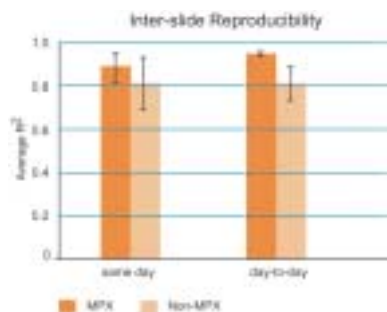
Nexterion™ Slide MPX is designed to allow the simultaneous analysis of multiple biological samples in one microarray experiment, with improved reproducibility and sensitivity at lower experimental costs when compared to conventional single-array slides.

The product range consists of coated glass slides that are partitioned into individual wells by an ultra-hydrophobic patterning layer. The slides are supplied together with removable superstructures (incubation chambers) and sealing strips in a ready-to-use kit. Cross contamination is avoided during multiplexed hybridization through the hydrophobic pattern material and the use of the superstructure.

It is great for multiplexed experiments, side-by-side comparisons, replicate experiments, providing an ideal platform for for toxico- and pharmacogenomic studies, SNP genotyping and CGH assays, cellular pathway analysis, agricultural research, and molecular diagnostics.

Description	Cat.#	Qty
Nexterion Slide MPX 16 wells	O6960	1u
Nexterion Slide MPX 48 wells	O6970	1u
Dimensions : 75.6 mm x 25.0 mm +/- 0.2 mm Thickness : 1.0 mm +/- 0.05 mm Wells area : 6.59 x 6.59 mm (16 wells) - 2.5 x 2.5 mm (48 wells)		

D.118



Following coating chemistries are available (please inquire for ordering) :

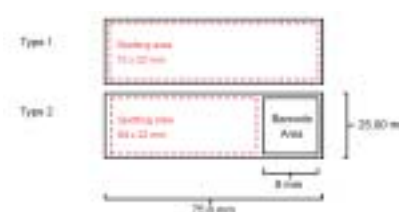
- Aminosilane (A and A+)
- Epoxysilane (E)
- Aldehyde (AL)
- Hydrogel (H)

Nexterion™ Slide AL

Nexterion Slide AL is especially suitable for covalent immobilization of PCR products, cDNA molecules, but also for synthetic oligonucleotides. An amino-modification is recommended for efficient immobilization of oligonucleotides. In addition, peptides, proteins, antibodies, cells and tissues can be immobilized on Slide AL. The aldehyde group will reversibly react with NH₂-groups to form a Schiff's Base. Reduction with sodium borohydride will result in the irreversible immobilization of the nucleic acids and inactivation of unreacted aldehyde groups.

Description	Cat.#	Qty
Nexterion™ Slide AL, Starter Kit Includes : 10 Nexterion Slide AL, 10 ml Nexterion Spot, 100 ml Blocking Solution, 10 ml Nexterion Hyb	T50391	each
Nexterion™ Slide AL, 75.6 mm x 25 mm	U91873	25 slides
Nexterion™ Slide AL, 75.6 mm x 25 mm bar-coded	T78503	25 slides

For the spotting and binding of DNA-probes onto Nexterion™ Slide AL we recommend the optimized spotting buffer Nexterion™ Spot (#T78512). The latter is especially useful to avoid evaporation of the spotting solution during the spotting process. Blocking is recommended with NaBH₄, and hybridization with Nexterion Hyb solution (#U65433).



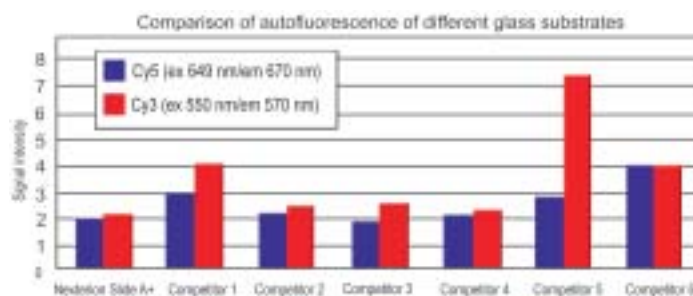
Nexterion™ Slide A+

Nexterion™ Slide A+ is especially suitable for efficient immobilization of PCR products, cDNA molecules and longer, synthetic oligonucleotides (size : 50 mers). The product properties are optimized to support reliable microarray hybridization results and data analysis by providing high signal intensities and excellent spot morphology.

Nexterion Slide A+ is compatible with most common protocols for aminosilane slides, giving users the opportunity to employ their established microarray processes. It is an ideal substrate, which allows scientists to change PCR product and/or cDNA probes to longer oligonucleotide probes without changing the surface chemistry and protocol. A re-optimization of the entire process for the oligonucleotide applications is not necessary. Thus Nexterion Slide A+ allows an economic transfer not only from PCR products and/or cDNA molecules to longer oligonucleotides, but also from any aminosilane slide to Nexterion Slide A+.

Description	Cat.#	Qty
Nexterion™ Slide A+, 75.6 mm x 25 mm	U76362	25 slides
Nexterion™ Slide A+, 75.6 mm x 25 mm barcoded	U76372	25 slides

Nexterion™ Slide A + is compatible with most common spotting solutions. The betaine or DMSO containing spotting solutions are especially suitable when probe evaporation during long spotting runs is expected Nexterion™ spot (#T78512) Blocking is recommended with BSA or succinic acid and hybridization with Nexterion Hyb solution (#U65433).



Nexterion™ Spotting solutions

We provide high quality reagents for your hybridization experiments with Nexterion slides. Experiments show best results using both Nexterion slides and the nexterion solutions and buffers as recommended in previous description of slide.

Nexterion™ Spotting solution

Our spotting buffer **Nexterion™ spot** (#T78512) is optimized for the spotting and binding of DNA-probes onto Nexterion slide E, Nexterion slide AL and Nexterion slide A+. Based on phosphate buffer with an alkaline pH, they ensure efficient immobilization and homogeneous spots, in comparison with conventional solutions (see technical tip) which are also provided as high quality reagents.

- ◆ Smaller and more homogeneous spots
- ◆ Efficient immobilization

Technical tip

Nexterion spot compared with conventional buffers

Spotting Solution	Remark
50 % DMSO	Larger spot size, prevents evaporation problems during long spotting runs
3 x SSC	Smaller spots, standard aqueous spotting solution
3 x SSC + 1.5 M betaine	Larger spots, prevents evaporation problems during long spotting runs, very homogeneous spots
Nexterion™ Spot	Smaller spots and more homogeneous spots, efficient immobilization

Description	Cat.#	Qty
Nexterion Spot (2 x) Based on phosphate buffer with an alkaline pH. Ensures efficient immobilization and smaller homogeneous spots. Recommended for Nexterion slides E and AL.	T78512	100 ml
DMSO DNase, RNase free Used at 50 % concentration. Larger spot size. Prevents evaporation problems during long spotting runs.	BB3970	250 ml
Betaine HCl Used at 1.5 M with SSC 3x to prevent evaporation during long term spotting runs. However, may give larger spots.	N15120	1 kg
Formamide Used at 25 % concentration in FBNC buffer for Nexterion Slides A+.	070990	100 ml
Nitrocellulose Used at 0.5 µg/µl concentration in FBNC buffer for Nexterion Slides A+.	AT7810	250 g
SSC buffer (20x) High quality (made from purest grade materials), micro-filtered. Used at 3x concentration with all Nexterion slides.	UP586046	4 L (80 L)
SSC ready-pack (1 pack to prepare 1 L of 20x solution) Same, but in convenient packs (reduced volume storage, increased stability).	674080	2 pk (40 L)

Nexterion™ Blocking solutions

Slide Nexterion™ Block E (for Nexterion Slide E)

For blocking arrays printed on Nexterion Slide E we recommend our Nexterion Block E :

- ◆ Optimal blocking performance.
- ◆ Higher reproductibility and quality.

Description	Cat.#	Qty
Nexterion™ Block E (4x)	U76353	100 ml
Nexterion™ Block E (4x)	U76354	1000 ml

*Should be diluted 1:4 with water prior to the stocking process.

Technical tip

After spotting it is important to remove unbound DNA-molecules and buffer substances from the slides by extensive washing avoiding then any interference with subsequent hybridization experiments. Consequently, we recommend to block the slides before hybridization step.

The blocking solution contains molecules that rapidly react with residual covalent binding sites in both, the printed and unprinted areas of the slide surface. Failure to totally block reactive groups may result in non-specific binding of labeled target and probe molecules to the slide surface.

This potentially irreversible covalent coupling of the labeled target is the first component of the non-specific background problem. The second component is weak adhesive association (hydrophobic or electrostatic) of the labeled target with the slide surface. Both cause a fluorescent background, which can be eliminated or substantially decreased through the use of Nexterion Block E.

Blocking solution for Nexterion™ Slide AL

For blocking arrays printed on Nexterion Slide AL we recommend to treat the exposed aldehyde groups with sodium borohydride (NaBH₄) thereby reducing them to hydroxyl groups.

The aldehyde blocking solution for approximately 5 slides contains :

- ◆ 1.5 g NaBH₄ (# 07998U)
- ◆ 450 ml PBS (phosphate buffer saline, # 68723A)
- ◆ 133 ml 99 % ethanol (to reduce bubbling)

Blocking solution for Nexterion™ Slide A+

After immobilization it is important to remove unbound DNA molecules and buffer substances from the slides by extensive washing avoiding then any interference with subsequent hybridization experiments. Additionally, blocking free functional groups is required to avoid non-specific binding. Both are achieved by following one of the two protocols given in the respective manual for processing Nexterion Slide A+. Protocol A is based on a pre-hybridization in the presence of BSA whereas protocol B uses blocking with succinic anhydride.

Amino slide pre-hybridization solution (protocol A with pre-hybridization) :

Amount sufficient for up to 5 slides

- ◆ 25 ml Nexterion™ Hyb (#U65433)
- ◆ 25 ml H₂O Rnase/Dnase free (# 457420)
- ◆ 500 mg BSA Rnase/Dnase free (# 909380)

Amino slide blocking solution (protocol B with blocking step) :

Amount sufficient for up to 20 slides

- ◆ 5 g succinic anhydride (# 04746E)
- ◆ 315 ml 1-Methyl-2-pyrrolidone
- ◆ 35 ml 0.2 M sodium borate (# 773280)

Nexterion™ Hybridization solutions

The hybridization of Nexterion™ Slide E, Nexterion Slide AL and Nexterion Slide A+ can be done in commercially available hybridization chambers or with cover slips.

The purified and labeled target nucleic acid should be dissolved in Nexterion Hyb. The amount of buffer depends on the desired final nucleic acid concentration and also on the size of hybridization chamber used. In the case where the target nucleic acid is already dissolved in a different buffer or in water the sample can also be diluted in Nexterion Hyb. The concentration of Nexterion Hyb needs to be **at least 90% (v/v)** in the final hybridization solution (mixture ratio 1:9).

Our Nexterion Hyb increases the microarray hybridization reactions efficiency by expediting base pair formations between complementary target sequences that are attached to the microarray surface and labeled probe molecules in solution. Our Nexterion Hyb also reduces background fluorescence.

The buffer components stabilize extended hybridizations. The buffer is compatible with many different surface chemistries and is ready to use.

Description	Cat.#	Qty
Nexterion™ Hyb	U65433	100 ml
Sodium borohydride (NaBH ₄)	07998U	100 g
PBS, powder	68723A	for 10 L
Water, nuclease free, sterile	457420	500 ml
Albumin Bovine Fraction V, Highest Purity	909380	5 g
Sodium borate	773280	1 kg

Macro and Micro-Array

Slides

Genorama™ MicroArraying

Genorama™ Microarray Slides are developed and tested primarily for DNA-based applications. However, RNA and proteins can also be immobilized.

Genorama™ Microarray Slides

Characteristics :

- ◆ Double-side coating with a printable area of 23 x 73 mm.
- ◆ Very clean and uniform surface
- ◆ Long storage (>6 months at +4°C)
- ◆ 2 surfaces coatings available :
 - SA activated slides (optimized for gene expression)
 - SAL activated slides (optimized for genotyping)
- ◆ 2 sizes available :
 - Standard 25 x 75 x 1 mm
 - Thin slides 24 x 60 x 0.15 mm*

Quality :

The activated glass slides are produced under rigorous quality control and tested for low background and good immobilization characteristics. As a result, the slides have a very clean and uniform surface, highly suitable for printing top quality microarrays. 24 x 60 mm slides have one specially refined edge to ensure maximum uniformity of light distribution via total internal reflection excitation in the Genorama 003 Imaging System.

Coated slides are packed in boxes of 25 and sealed in an alufoil bag to protect the activated surface from light and maintain a stable storage environment. Bar-coded slides are available upon request.

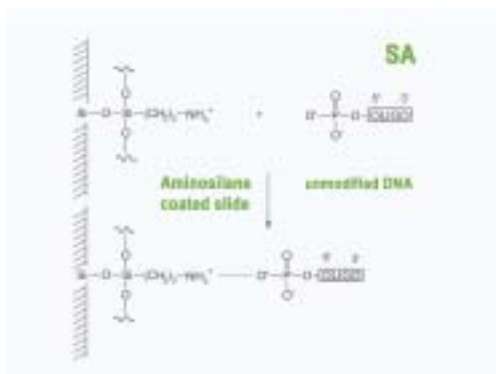
The process is certified to be in accordance with ISO9001 standards.



Type	Genorama™ SA Slide		Genorama™ SAL Slides	
Coating	Aminosilane		Aminosilane with linker	
For binding	Unmodified DNA, long aminated DNA (with Edman reagent)		Short aminated DNA	
Type of binding	Non-covalent bound		Covalent bound	
Optimized for	Gene expression		Genotyping	
Buffer	Genorama™ Spotting Solution II		Genorama™ Spotting Solution I	
Items				
Standard size	BB3730	25 slides (25 x 75 x 1 mm)	BB3760	25 slides (25 x 75 x 1 mm)
Thin size	BB3750	25 slides (24 x 60 x 0.15 mm)	BB3770	25 slides (24 x 60 x 0.15 mm)

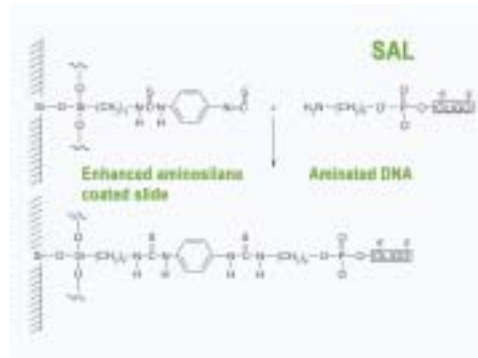
SA- Slides

Slides are coated with 3-Aminopropyltrimethoxy-silane. Unmodified oligos bind to aminosilane electrostatically coated slide. Oligos can be attached either at their 5' termini or via internal phosphate groups (backbone).



SAL- Slides

Slides are coated with 3-Aminopropyltrimethoxy-silane + 1,4-Phenylenediisothiocyanate. Aminated DNA attaches via its 5' terminate to 3-Aminopropyltrimethoxysilane + 1,4-Phenyl-enediisothiocyanate coated glass surface by formation of covalent bond.



GENORAMA™ Spotting Solutions

Genorama™ Spotting Solutions are optimized and highly recommended together with the microarray slides SAL and SA in order to ensure proper quality.

- ◆ Ready-to-use
- ◆ convenient :
 - 2x stock solution
 - in bottles of 50 ml and 100 ml
- ◆ Cost-effective
- ◆ ISO 9001



Type	Genorama Spotting Solution I	Genorama™ Spotting Solution II
Optimized	Genorama™ Microarray Slides SAL	Genorama™ Microarray for slide Slides SA
Cat.#	BA9811 50 ml BA9812 100 ml	BB3550 50 ml BB3551 100 ml

Microarray preparation also needs oligos, and cloning/PCR reagents...!

ProPlate™ multi-array

Proplate™ is an integrated microscope multi-array slide module suitable for screening samples from small scale to automated HTS, as well as for QC of slides or optimization of DNA labeling protocols. It can be used to process cDNA or oligonucleotide arrays.

- ◆ 7 x 7 mm square wells are ideal for high content arrays.
- ◆ 15 - 350 µl well volumes accommodate small reagent and large wash volumes.
- ◆ Form up to 16 leak-proof wells on any slide surface.
Removable seal strips close easily wells for incubations (no use of adhesive). The standard microtiter well spacing facilitates use with multichannel pipettes.
- ◆ Four slides fit into a tray, producing a modular plate fitting to a standard microtiter plate footprint, allowing automated robotic processing of 64 arrays.

Description	Cat.#	Qty
ProPlate™ multi-array slide module Includes disposable 16 wells module and 2 clips	FP-BC4793	2 u
ProPlate™ multi-array system Includes four 16-well slide modules, one tray and cover, ten seal-strips and one applicator	FP-BC4781	1 set
ProPlate™ adhesive seal-strips Includes 50 removable covers and 1 applicator	FP-BC4801	1 set
ProPlate™ tray and cover Includes one tray and one cover	FP-BC4811	1 set

Ask for High Efficiency Competent cells, and DNA polymerases !



Slides can be assembled easily for processing and disassembled quickly for reading with conventional chip readers due to adhesive free, snap-clip design.



Removable Seal-Strips facilitate mixing and eliminate evaporation.

