Technical Instructions for Spotting Microarrays

PRODUCT

Nexterion™ Slide A is an activated glass slide in the standard size 75.5 mm x 25 mm x 1.0 mm with a multi-amino-silane* surface coating for efficient binding of cDNA molecules, PCR products and longer oligonucleotides (size ≥ 50 mers).
*patent pending

DURABILITY AND STORAGE

Use by shelf-life date if stored in sealed condition. Open and use the slides in a clean environment to avoid particle build-up on the printing surface. Avoid direct contact with the printing surface to minimize contamination and abrasion of the coated surface. After opening the package, store the remaining slides in a dust-free, light protected, and dry area at room temperature and use within two weeks.

ARRAY PRINTING

1. Mix equal amounts of oligonucleotide probe or PCR product and 50% DMSO to obtain a minimum final probe concentration of 20 µM for oligos, or 0.3 mg/ml for PCR products. For smaller spot sizes, 3X SSC can be used as a printing buffer.
2. Transfer an appropriate volume of probes to a microtiter plate.
3. Set up the arrayer according to the manufacturer's recommendation and print slides at 40-50% relative humidity.

DNA IMMOBILIZATION

1. Rehydrate slides over a boiling water bath for 2-3 seconds, and then snap-dry for 10 seconds at 85°C on a hot plate.
2. UV cross link at 600 mJ for PCR products or 800 mJ for oligonucleotides.
3. Proceed immediately to prehybridization (washing and blocking) or store the arrays in a desiccator for no more than two weeks.

<OPTIONAL> ARRAY DENATURATION (only for PCR products)

1. 1 x 30 sec in 0.1% SDS at room temperature.
2. 1 x 3 min in deionised H_2O at 95°C.
3. 1 x 2 min 70% ethanol.
4. Spin-dry slides immediately at 200 x g for 5 min.

WASHING AND BLOCKING

1. 1 x 60 min Prehybridization Buffer (5X SSC, 0.1% SDS, 1.0% BSA, 50% formamide, 0.01% salmon sperm DNA) at 42°C.
2. 5 x deionised H_2O at room temperature.
3. Spin-dry slides immediately.

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HYBRIDIZATION

1. Re-suspend or dilute the labeled target in Prehybridization Buffer (5X SSC, 0.1% SDS, 1.0% BSA, 50% formamide, 0.01% salmon sperm DNA).
2. Denature the suspended target by heating at 95°C for 3 min and apply the appropriate volume onto the array surface of a blocked slide.

POST-HYBRIDIZATION AND WASHING

1. Wash 1 x 5 min in 2X SSC and 0.1% SDS at room temperature to remove the cover slip.
2. Wash 1 x 5 min in 2X SSC at room temperature.
3. Wash 1 x 1 min in 0.2X SSC at room temperature.
4. Wash 1 x 5 sec in 0.05X SSC at room temperature.
5. Dry the array in an oil free air or nitrogen stream or by centrifugation.

IMPORTANT INFORMATION ABOUT PATENTS

Using arrays based on SCHOTT Nexterion products for dual color analysis on a single array in which at least two different samples are labeled with at least two different labels may require a license under one of the following patents: U.S. patent nos. 5,770,358 or 5,800,992 or 6,225,625 and U.S. patent no. 5,830,645. Manufacturing and use of probe arrays may require a license under the following patents: U.S. patent no. 6,040,138 or 5,445,934 or 5,744,305 and under the following patents owned by Oxford Gene Technology Ltd. („OGT"): European patent no. EP 0,373,203, U.S. patent nos. 5,700,637 and 6,054,270 and Japanese patent nos. 3393528 and 3386391 ("The OGT patents").
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