

Technical Instructions for Spotting Microarrays

PRODUCT

Nexterion™ Slide A+ is an activated glass slide in the standard size 75.6 mm x 25 mm x 1.0 mm with amino-groups for efficient binding of PCR-products, cDNA and long oligonucleotides (size ≥ 50 mer). An amino link is not required but do not interfere with immobilization when present.

DURABILITY AND STORAGE

Sealed 3 months at room temperature. After unsealing store dust-free, light-protected, under dry conditions at room temperature

ARRAY PRINTING

1. Dissolve oligonucleotide probe or PCR product in 3xSSC, 1.5 M betaine in 3x SSC, 50% DMSO or Nexterion spotting solution (Next Spot) to obtain the recommended final probe concentration (2 to 20 µM for oligonucleotides, 0.05 to 0.5 µM or 0.1 to 1.0 µg/µl for PCR products).
2. Transfer an appropriate volume of probes to a microtiter plate.
3. If required (white precipitation) heat probe to 50 - 80 °C prior to spotting for 2 min.
4. Setup the arrayer according to the manufacturers recommendations and print slides.

DNA IMMOBILIZATION

1. UV-cross link printed slides at 250 mJ
- and
2. incubate cross linked slides for at least 12 h at room temperature (over night).
- Intermediate storage if required (dust-free, light-protected, at room temperature and dry).

WASHING AND BLOCKING

Protocol with pre-hybridization, duration 50 min:

1. 1 x 10 to 20 sec in 0.1 % SDS at room temperature
2. 1 x 10 to 20 sec in dH₂O at room temperature
3. (only when PCR-products were spotted)
1 x 3 min in dH₂O at 95°C
4. 1 x 45 min in Pre-Hybridization Buffer (3 –5xSSC, containing 0.1% SDS and 0.1 mg/ml BSA or 25 ml Nexterion Hyb + 25 ml dH₂O + 500 mg BSA, volume for 5 slides) at 42°C
5. 1 x 10 to 20 sec in dH₂O at room temperature
6. Dry slides immediately

Alternative protocol with chemical blocking , duration 20 min:

1. 1 x 15 min in Amino Blocking Solution (5 g succinic anhydride + 315 ml n-methylpyrrolidone + 35 ml 0.2 M sodium-borate pH 8, add sodium-borate freshly before use) at room temperature
2. 1 x 10 to 20 sec in 0.1 % SDS at room temperature
3. 1 x 10 to 20 sec in dH₂O at room temperature
4. (only when PCR-products were spotted)
1 x 3 minutes in dH₂O at 95°C
5. Dry slides immediately

Volume of washing solution: at least 250 ml for 5 slides. Final drying of slides in oil-free air or nitrogen stream or centrifuge 150 to 200x g for 2 min. The slides should never dry between washing steps (keep transfer times into next liquid < 30 sec).

HYBRIDIZATION

1. Re-suspend or dilute the labeled target in Nexterion Hyb (formamide-free, mixture ratio sample: buffer 1:9) or commonly used SSC based buffers, i.e. 3 to 5xSSC, 0.1% SDS with and without formamide and competitor 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 WASHING

1. Wash 1 x 10 min in 2X SSC and 0.2% SDS at room temperature.
2. Wash 1 x 10 min in 2X SSC at room temperature.
3. Wash 1 x 10 min in 0.2X SSC at room temperature.
4. Dry the array in an oil free air or nitrogen stream or by centrifugation at 150 to 200x g for 2 min to avoid water stains on the slide surface.

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 6,625,225 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"). Other patents may apply. The purchase of Nexterion™ products does not convey any license under any of the OGT patents or any of the other patents referred to. For all applications SCHOTT North America Inc. and SCHOTT Nexterion AG make no representation or warranty that the practice of its technology and products or any improvement will not infringe or violate any domestic or foreign patent of any third party. To inquire about licensing under the OGT patents, please contact OGT at licensing@ogt.co.uk.

Complete protocol, additional information and online-ordering at:

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