

Protocol



Nexterion® Slide AL Evaluation Kit DNA application

Dok-Nr.:	LS6-HBM-M-002
Version:	1.2
Seite:	1/4
Datum:	© April 2009

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1 Introduction

Important Note for the Use of additional Spotting Solutions and Oligonucleotides provided in the Nexterion[®] Slide AL Evaluation Kit

This note describes the use of the three spotting solutions and the probe and target oligonucleotides provided within this Nexterion[®] Slide AL Evaluation Kit. For a detailed description of Slide AL processing including all blocking and washing steps, please refer to the current Slide AL protocol.

2 Material provided with the Kit

Items	Description	Storage at
Probe-Oligonucleotide	2 nmol Amine-modified oligonucleotide for spotting	-20 °C
Target-Oligonucleotide	3 pmol Cy3-labeled oligonucleotide for hybridization	-20 °C
Nexterion [®] Slide AL	Aldehydesilane-coated Slides	+20 to 25 °C
Nexterion [®] Spot	Spotting Solution (2x concentrated)	+20 to 25 °C
Nexterion [®] Hyb	Hybridization Solution (ready-to-use)	+20 to 25 °C

3 Spotting solutions


Spot size and spot morphology is influenced by a number of parameters, among them are

- Environmental conditions like relative humidity and temperature
- Spotter/spotter settings and pins (material, diameter etc.)
- Slide surface
- Spotting solution
- Quality and concentration of probe DNA

To reduce the variability of spot size and spot morphology, it is important to carefully control and optimize the spotting process. To assist in this process, we provide a spot buffer that is suitable for most applications. However, if the spot size or morphology is not satisfying, please contact our Technical support for further information.

The spotting solutions are 2 x concentrated and can be diluted by adding an equal volume of diH₂O before dissolving the probe DNA (oligonucleotides or PCR products) for spotting.

Alternatively, the probe DNA can be first dissolved in diH₂O to get a 2 x concentrated stock solution. Aliquots of this stock solution can then be combined with equal volumes of the respective 2 x concentrated spotting solutions.

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4 Probe-oligonucleotides for spotting

The recommended final concentration of probe-oligonucleotide is between 10 and 20 μM .
Preparation of a 20 μM probe-oligonucleotide solution:

1. Dissolve the probe-oligonucleotides provided with the Kit in 50 μl of diH_2O to get a final concentration of 40 μM . As oligonucleotides may not dissolve readily in diH_2O , ensure the samples are thoroughly mixed. For long-term storage, oligonucleotides should be stored dry at $-20\text{ }^\circ\text{C}$.
2. Prepare three fresh tubes and add 10 μl of the oligonucleotide stock solution to each tube.
3. Add 10 μl of the respective 2 x spotting solution provided with the Kit and vortex. The final concentration of probe-oligonucleotides for spotting is 20 μM . The probes are now ready for printing.
4. For details of printing and immobilization see Nexterion[®] Slide AL protocol.

5 Target-oligonucleotides for hybridization

The amount of target-oligonucleotide provided (3 pmol) is intended to be used for the hybridization of 3 slides with 100 μl hybridization solution per slide containing 10 nM labelled target-oligonucleotide. If more hybridization solution has to be prepared, the target can be diluted further using Nexterion[®] Hyb to get a final concentration of target-oligonucleotide as low as 1 nM. This concentration will also yield signal intensities sufficiently high enough for analysis.

Preparation of a 10 nM target-oligonucleotide solution:


Re-suspend the Cy3-labeled target-oligonucleotide in 300 μl Nexterion[®] Hyb. This yields a final concentration of 10 nM. This solution can be used directly for hybridization.

Incubate the suspended target by heating at $95\text{ }^\circ\text{C}$ for 3 min in a water-filled well of a heat block, perform a quick spin in a microcentrifuge, then pipette the appropriate volume onto the array surface of a blocked slide under the coverslip or inside a hybridization station.

6 Hybridization

Incubate the slides at $42\text{ }^\circ\text{C}$ for 1 hour.

For further details of processing slides see the Nexterion[®] Slide AL protocol.

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7 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"). 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 Jena^{er} Glas GmbH 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. Before making or using any oligonucleotide arrays you should contact OGT to discuss a licence. To inquire about licensing under the OGT patents, please contact OGT at licensing@ogt.co.uk.