

Protocol

SCHOTT

Nexterion® Epoxy Slide Oligo Processing Kit DNA application

Dok-Nr.:	LS6-HBM-N-002
Version:	1.0
Seite:	1/4
Datum:	© Jan 2010

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1 PRODUCT OVERVIEW

SCHOTT Microarray Solutions offers a kit with pre-prepared reagents for the blocking, hybridization and washing of 25 printed epoxysilane coated slides. The reagents in the kit are optimized for use with epoxy coated slides (e.g. Nexterion® Slide E) printed with 20 to 70 mer oligonucleotides. The Nexterion® Oligo Pre-Hyb offers the advantage combining some of the initial washing and blocking steps thereby reducing the overall processing time. Nexterion® Oligo Hyb contains formamide, which allows for increased hybridization stringency at decreased hybridization temperatures. The kit includes an easy to- use slide processing protocol.

2 KIT CONTENTS

Kit components	Description	Store at	25 Slide Kit	100 Slide Kit	500 Slide Kit
Nexterion® 70mer Oligo Pre-Hyb	Pre-hybridization Solution (ready-to-use)	4°C	1x500 ml	3x500 ml	5x1000 ml
Nexterion® 70mer Oligo Hyb	Hybridization Solution (ready-to-use)	4°C	1x10 ml	2x10 ml	1x100 ml
Nexterion® 70mer Oligo Wash A	Washing Solution Component A	20 to 25°C	1x1000 ml	2x1000 ml	6x1000 ml
Nexterion® 70mer Oligo Wash B	Washing Solution Component B	20 to 25°C	1x100 ml	3x100 ml	2x1000 ml

3 STORAGE AND HANDLING

Store the Nexterion® Pre-hybridization Solution and the Nexterion® Hybridization Solution at 4°C and use prior to the expiration date.

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4 PRE-HYBRIDIZATION

Note: Do not allow the slides to dry during the pre-hybridization and washing procedure.

1. Pre-warm an appropriate volume of Nexterion® 70mer Oligo Pre-Hyb to 42°C for several minutes until the precipitate is completely dissolved.
2. Incubate slides in Nexterion® 70mer Oligo Pre-Hyb for 60 min at 42°C. Use a large enough volume of buffer to ensure that all slides are completely covered with Nexterion® Pre-Hyb.
3. During the pre-hybridization step, prepare Wash Solution 1 by diluting Nexterion® 70mer Oligo Wash B 1:40 with diH₂O. Prepare 50 ml per slide (i.e. 1.25 ml Nexterion® 70mer Oligo Wash B, add diH₂O to 50 ml).
4. Wash 5 min in Wash Solution 1 at 20 to 25°C.
5. Rinse with diH₂O for 30 sec.
6. Dry the Nexterion® Slide E in either an oil-free air (or nitrogen) jet or by centrifugation (200 x g for 5 min). This will avoid leaving any drying marks on the slide surface.

5 HYBRIDIZATION

1. Re-suspend the dried, labeled target to be applied to the array in Nexterion® 70mer Oligo Hyb. If the target is already dissolved in a different buffer or water, the sample can also be diluted in the hybridization solution to get at least 90% (v/v) buffer in the final hybridization solution.
2. Denature the re-suspended target by heating at 95°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 of hybridization solution containing the target onto the array surface of a pre-hybridized slide under the coverslip or inside a hybridization station.
3. It is suggested to hybridize the slide at 42°C for 16 hours. For further details, please read the notes in the appendix.

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6 POST- HYBRIDIZATION WASHING

Note: Do not allow the slides to dry during the hybridization and washing procedure. Wash Solution 2, 3 and 4 should be prepared in advance. Use at least 50 ml solution per slide.

Post-hybridization washing solutions	Preparation
Wash Solution 2	50 ml Nexterion 70mer Oligo Wash A, 25 ml Nexterion 70mer Oligo Wash B, Make up to 500 ml with diH ₂ O
Wash Solution 3	50 ml Nexterion 70mer Oligo Wash A, Make up to 500 ml with diH ₂ O
Wash Solution 4	5 ml Nexterion 70mer Oligo Wash A, Make up to 500 ml with diH ₂ O

1. Place the array into a slide rack and immerse in a dish containing Wash Solution 2. Wash in the above solution 1 x 10 min at 20 to 25°C.
2. Wash 1 x 10 min in Wash Solution 3 at 20 to 25°C.
3. Wash 1 x 10 min in Wash Solution 4 at 20 to 25°C.
4. Dry the array either in an oil-free air (or nitrogen) jet or by centrifugation (200 x g for 5 min).
5. Protect the array from light, dust and abrasion of the array surface, until ready for scanning. Ensure that the laser and filter set of the scanner are compatible with the fluorescent labeling of the probe molecules.