

Protocol



Nexterion® Plate A+ MTP 96 DNA-application

Dok-Nr.:	LS6-HBM-M
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1 Product overview

Nexterion[®] Plate A+ MTP 96 was developed for the fast and efficient immobilization of DNA molecules onto a coated microplate sized glass plates allowing high sample throughput with superior reproducibility and higher sensitivity. The plate is manufactured from the highest quality glass and laser cutting techniques were used to obtain defect and particle free plate surfaces with excellent dimensional tolerances. Nexterion[®] Plate A+ MTP 96 are cleaned and coated under cleanroom conditions and the strict process control during all the fabrication steps ensures excellent coating uniformity and batch-to-batch reproducibility.

The amino surface coating allows for the efficient covalent binding of molecules, such as PCR-products, cDNA molecules and longer, synthetic oligonucleotides (size ≥ 50 mer). The uniform and ultrahydrophobic patterning material separates the plate surface into 96 individual wells. Nexterion[®] Plate A+ MTP 96 is available with a self-adhesive 96 well superstructure and sealing film to ensure excellent sample mixing, and minimal cross contamination and sample evaporation during the hybridization steps.

2 Storage and handling

1. Store the substrates in unopened packaging at room temperature (20 - 25 °C) and use prior to the expiration date.
2. Open and use the substrates in a clean environment to avoid particle build-up on the printing surface.
3. Avoid direct contact with the printing surface to minimize contamination and abrasion of the coated surface.
4. Once the package has been opened, substrates should be used within 2 weeks.
5. Only one side of each plate is patterned and must be used with the pattern facing up. The pattern corner chamfer corresponds to the A1 position in the tray.

3 General precautions

1. The protocols contained in this document are meant to be general guidelines only and some optimization may be required depending on the application and sample being used.
2. Refer to manufacturer supplied Material Safety and Data Sheets (MSDS) for proper handling and disposal of all chemicals.
3. Nexterion[®] Plate A+ MTP 96 is for research use only, not for *in vitro* diagnostic use.

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4 Reagents required

1. Deionized water (diH₂O)
2. 2 x Nexterion® Spot Solution or 3 x SSC, 3 x SSC containing 1.5 M betaine or 50 % DMSO
3. Hybridization Buffer Nexterion® Hyb (formamide-free) or 3 - 5 x SSC + 0.1 % SDS with or without competitor DNA and formamide
4. Saline sodium citrate (20 x SSC) - Ambion 9673
5. Sodium dodecyl sulfate (SDS) - Fisher BP166-500 or 10 % SDS solution for washing (10 g sodium dodecyl sulfate in 100 ml diH₂O, dissolve at room temperature)
6. 0.1 % SDS (10 ml 10 % SDS solution in 1000 ml diH₂O)
7. Pre-Hybridization Buffer (3 - 5 x SSC, containing 0.1 % SDS and 0.1 mg/ml BSA) or alternatively 25 ml Nexterion® Hyb + 25 ml diH₂O + 500 mg BSA (volume for 1-2 plates)

5 Equipment required

1. UV cross-linker (Stratagene Stratalinker).
2. Heat block capable of heating to 95 °C.
3. Heated water bath.
4. Centrifuge with plate holders or compressed nitrogen gas for drying slides.
5. Plate dish and rack combo (Fisher 900200) for washing plates.

6 Array printing

1. Dissolve oligonucleotide probe or PCR product in the appropriate spotting solution to obtain the recommended final probe concentration:

DNA Probes	Final Spotting Concentration
Oligonucleotides	2 - 20 µM
PCR Products	0.05 - 0.5 µM (approx. 0.1 - 1 mg/ml)

Spotting solutions commonly used for Nexterion® Slide A+ MPX 16:

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, phosphate buffer based solution

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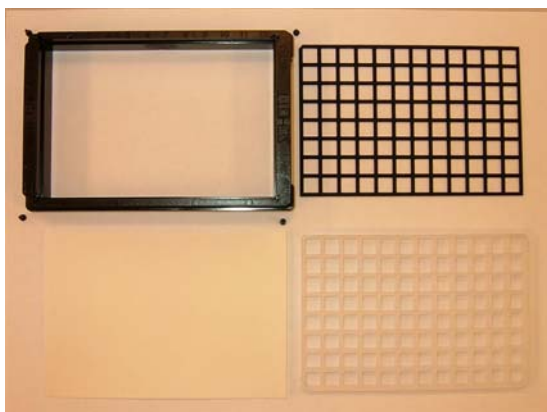
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2. Transfer an appropriate volume of probes to a microtiter plate.

Note: DNA-probes in Nexterion® spotting solution can be stored at -20 °C until spotting. If the probe solution shows a white precipitation prior to spotting, heat the probes to 50 to 80 °C for 2 min and avoid any change of concentration by condensation.

3. Setup the arrayer according to the manufacturer's recommendations. If you were previously using slides that were thicker than 1.0 mm, for optimal spotting you may need to re-calibrate the distance between the slide surface and the spotting pins.
4. Print substrates at 40 - 50 % relative humidity at 20 to 25 °C.




Step 1: MTP kit components (clockwise: tray with 4 pins, patterned plate, superstructure & sealing film).



Step 2: Insert patterned plate upright into tray cavity with chamfered corner in the A1 position and secure with the 4 pins, one in each corner.



Step 3: Plate assembled and ready for printing.

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7 Printing guidelines

Nexterion® Plate A+ MTP 96 is compatible with all microarray printing and spotting methods, including contact and non-contact printing technologies.

The following table displays the maximum number of probes per subarray well for Nexterion® Plate A+ MTP 96 based on theoretical calculations using various pitch distances and types of source plate. The color-coding in the table indicates, for each pin configuration, the number of pins used for intra-well printing.

Detailed printing guidelines are available online at:
www.us.schott.com/nexterion

Table 1

Pitch (microns)	96 Well Sourceplate		384 Well Sourceplate				
	Pin Configurations (1 x 1-2-3-4-6), (2 x 1-2-3-4-6)		(1 x 1)	(1 x 2-4-6-8-12)	(2 x 1)	(2 x 2-4-6-8-12)	(4 x 1)
100	3969	3969	2268	2268	1296	2268	1296
150	1681	1681	902	902	484	902	484
200	961	961	496	496	256	496	256
250	576	576	288	288	144	288	144
300	400	400	200	200	100	200	100
350	289	289	136	136	64	136	64
400	225	225	90	90	36	90	36
450	169	169	78	78	36	78	36
500	121	121	44	44	16	44	16
1 pin/MTP Well	buffer is 300 microns + 1/2 pitch						
2 pins/MTP Well							
4 pins/MTP Well							

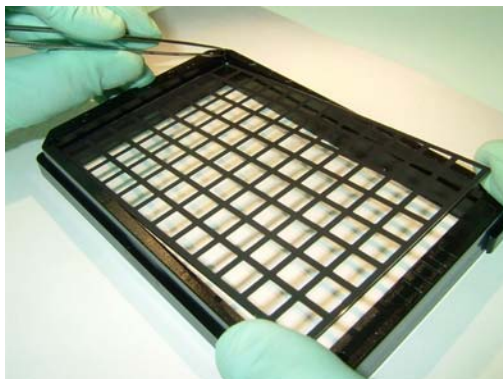
8 DNA immobilization

1. For covalent binding of DNA-probes on the slide surface after spotting it is necessary to UV-cross link the slides at 250 mJ.
2. Incubate the slides at room temperature for 12 h (e.g. store slides over night).
3. Proceed to Washing

Note: After spotting and immobilization, the arrays can be used immediately or stored under dry, dark conditions at room temperature for several month. The washing steps after immobilization should be carried out immediately before hybridization.

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Step 4: Printed plate disassembled for processing.

9 Washing and Prehybridization

After immobilization it is important to remove unbound DNA-molecules and buffer substances from the slides by extensive washing to avoid any interference with subsequent hybridization experiments. To avoid bleeding of the spots it is important to perform the washing steps very quickly by moving the slides (slide holder) up and down in the rinsing solution rather than using a shaker. The blocking is done by prehybridization with BSA.

1. 1 x 10 to 20 sec in 0.1 % SDS at room temperature
2. 1 x 10 to 20 sec in diH₂O at room temperature
3. (Denaturing step for arrays spotted with PCR-probes)
1 x 3 min in boiling diH₂O
4. 1 x 45 min in prehybridization buffer at 42 °C
5. 1 x 10 to 20 sec in diH₂O at room temperature
6. Dry the arrays in an oil-free air or nitrogen stream or by centrifugation (200 x g for 5 min) to avoid any water stains on the slide surface.

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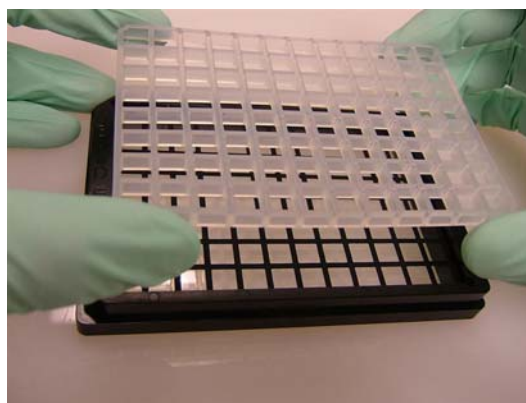
10 Hybridization

Assemble the processed microarray plate (steps 5 through 7) for hybridization.

PLATE ASSEMBLY



Step 5: Processed plate assembled.



Step 6: Remove the adhesive backing and apply superstructure to plate by holding it in 4 corners. Once the superstructure is aligned on the plate, press down on each individual well for 3-5 sec to ensure proper adhesion.



Step 7: Processed microarray plate is ready for hybridization.

- a. Resuspend the dried, labeled sample that will be applied to the array in an appropriate amount of 1 x hybridization solution as made for the prehybridization step.

Note: At least 7.5 pmol of each target in 30 μ l (maximum volume 200 μ l) of hybridization buffer per well is recommended. If the sample is already in solution, then add appropriate amounts of each of the hybridization solution components so that the final concentration for each is the same as listed in Table 2.

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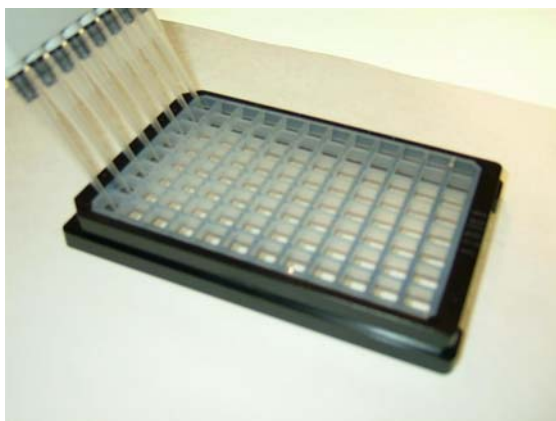
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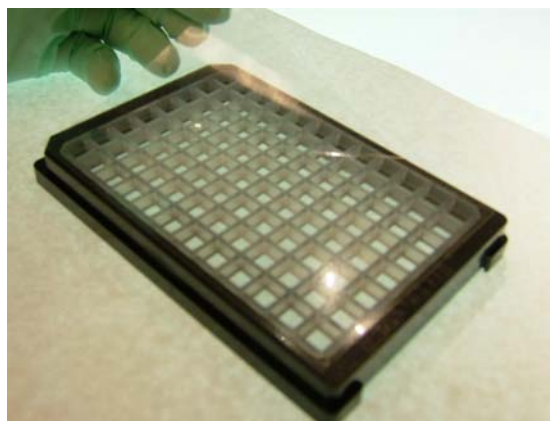
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- b. Denature the sample that will be applied to the array by heating it at 95 °C for 3 min in a water-filled well of a heat block, perform a quick spin in a micro-centrifuge, and pipette the appropriate volume into the individual wells of the plate (Step 8).

Caution: Placing the sample on ice after hybridization may cause the SDS to precipitate out of solution, which may interfere with hybridization. If the sample cannot be applied immediately after denaturation, place it in a 42 °C water-filled well of a heated block. Work rapidly when adding the target; use of filter tips is recommended:



Step 8: Add target using multi-channel pipette.



Step 9: Apply sealing film to cover entire well area.

2. Seal the wells immediately with the supplied sealing film (Step 9) ensuring that the seal makes good contact with the superstructure.
3. Hybridize the plates overnight at 42 °C if using formamide, or 50 - 70 °C if not using formamide on an orbital shaker at 100 rpm (Step 10). Protect the plates by covering with Aluminium foil.

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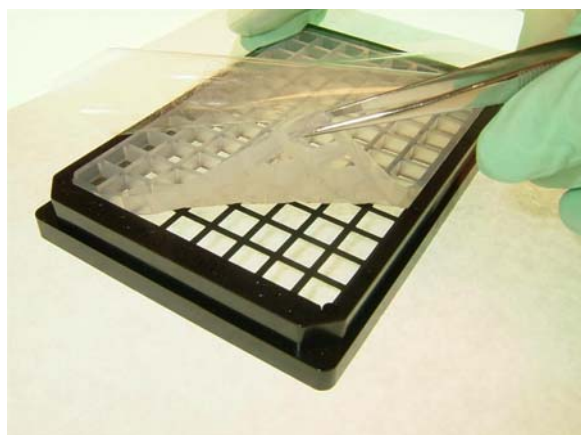
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11 Post-Hybridization washing

Caution: Do not allow plates to dry between washes, and protect them from light as much as possible. Never wash the plates with diH₂O after hybridization.

Note: The solutions recommended below for washing are a general guideline; your application may require alternative stringency washes. Wells can hold a maximum volume of 200 µl with superstructure attached.

1. Remove the sealing film and aspirate the target from the wells using a multi-channel pipette and immediately fill the wells with 100 µl of 2 x SSC. Aspirate the wash solution and fill the wells with fresh 2x SSC. Repeat this procedure 3 times.
2. Remove the superstructure immediately by peeling off from one corner. Ensure that the two sided adhesive film is removed with the superstructure. (Step 11)




Step 11: Remove seal strip, pin and superstructure after incubation.



Optional Step 12: Remove microarray plate from tray for washing

3. Immerse the plate (Step 12) in a suitable container containing 2 x SSC and 0.1 % SDS. Plunge gently 5 - 10 times.
4. Wash in 2 x SSC for 5 min at 42 °C, with gentle agitation.
5. Wash in 0.2 x SSC at room temperature for 1 min, plunging gently.
6. Wash in 0.05 x SSC at room temperature for 1 - 2 sec
7. Dry the arrays in an oil free air or nitrogen stream.
8. Add the clear plastic tray lid to protect the array surface from dust and abrasion. Keep the arrays away from light until ready for scanning.
9. If the plate was removed for washing, reassemble the plate into the tray holder with the 4 fixing pins prior to scanning.

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12 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 SCHOTT 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 Technical Glass Solutions 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.