Protocol

SCHOTT

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Nexterion® Plate E MTP 96

DNA-application

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For Technical Assistance, please contact

SCHOTT Technical Glass Solutions GmbH
Otto-Schott-Straße 13
07745 Jena
Germany

Phone: +49-(0)3641-681-4069
Fax: +49-(0)3641-681-4970
E-Mail: coatedsubstrate@schott.com

Additional information at:
www.schott.com/nexterion

1 Product overview

Nexterion® Plate E 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 E 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 epoxy surface coating allows for the efficient covalent and directed binding of molecules, such as oligonucleotides and/or PCR-products. Although use of amino-modified molecules is recommended, this modification is not essential. PCR-products or oligonucleotides react with the epoxy modified glass surface to form a covalent bond. The uniform and ultra hydrophobic patterning material separates the plate surface into 96 individual wells. Nexterion® Plate E 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 corner chamfer in the patterning 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 E MTP 96 is for research use only, not for *in vitro* diagnostic use.

4 Reagents required

1. Deionized water (diH_2O)
2. 2 x Nexterion® Spot Solution or 3 x SSC or low evaporation buffer (LEB)
3. Hybridization Buffer Nexterion® Hyb (formamide-free) or 3 - 5 x SSC containing 0.1 % SDS with or without competitor DNA and formamide
4. Saline Sodium Citrate (20 x SSC)
5. Sodium Dodecyl Sulfate (SDS)
6. Triton® X-100 solution (1 ml Triton® X-100 in 1000 ml diH_2O, dissolve by heating up to 60 °C to get 0.1 % solution)
7. HCl solution (Add 100 µl 37 % HCl to 1000 ml diH_2O to get 1 mM concentration)
8. KCl solution (Dilute 100 ml 1 M KCl stock solution to 1000 ml diH_2O to obtain 100 mM concentration)
9. Nexterion Blocking Solution (Dilute 100 ml 4 x Nexterion® Block with 300 ml diH_2O and add 80 µl of 37 % HCl to get 1 x solution)

5 Equipment required

1. UV crosslinker (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 plates.
5. Plate dish and rack combo (Fisher 900200) for washing plates.

6 Array printing

1. Mix equal amounts of oligonucleotide probe or PCR product and 2 x Nexterion® Spot Solution to obtain a recommended final probe concentration according to the following table:

DNA Probes	Final Spotting Concentration
Oligonucleotides	10 - 20 µM
PCR-products	0.1 - 0.5 µM (approx. 0.2 – 1 mg/ml)

Notes:

a) Use of the Nexterion® Spot Solution or LEB is recommended especially when spotting oligonucleotides.

b) To increase the spot sizes, detergents (SDS, Cetyl-trimethylammoniumbromid, Sarcosyl or Tween® 20) may be added to Nexterion® Spot or LEB, with a final concentration between 0.001 % and 0.1 % in the 2 x solution.

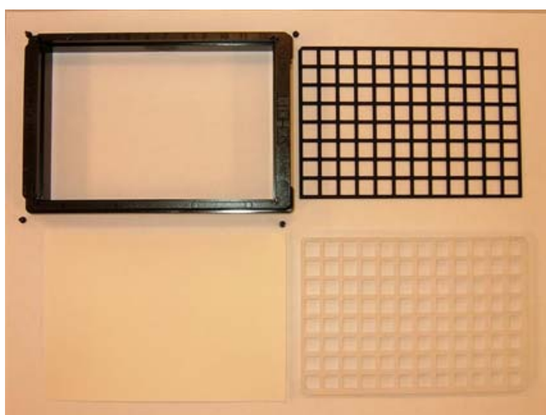
d) Alternatively, 3 x SSC or 3 x SSC containing 1.5 M betaine (final concentration) can be used as spotting buffers.

e) Do not use any spotting solution containing primary amino-groups like Tris.
f) PCR-products amplified with amino-modified primers are preferred for spotting. However, unmodified PCR-products can be also immobilized, because of their exo-cyclic amino-groups.
g) When an amino-functional primer is used to generate the PCR-products, the unused primers should be separated from the PCR-products prior to spotting.
h) Amino-modified oligonucleotides are immobilized more efficiently than unmodified oligonucleotides.

2. Transfer an appropriate volume of probes to a microtiter source plate.
Set up the arrayer according to the manufacturer's recommendations. The arrayer will need to be modified to take into account the height (10 mm) of the glass plate surface in the tray above the arraying platform. This will involve adjusting the distance the pin tool moves towards the plate surface known as Z-height calibration, target height, slide or surface height depending on the arrayer.

Note:
DNA-probes in Nexterion® Spot 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. Assemble the plate (steps 1 through 3) and print the substrates at 40 - 50 % relative humidity and 20 – 25 °C, then allow the arrays to air-dry for 10 min. Alternatively, (if the arrayer allows it), the glass plate may be printed directly onto without assembly into the tray.



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.

7 Printing guidelines

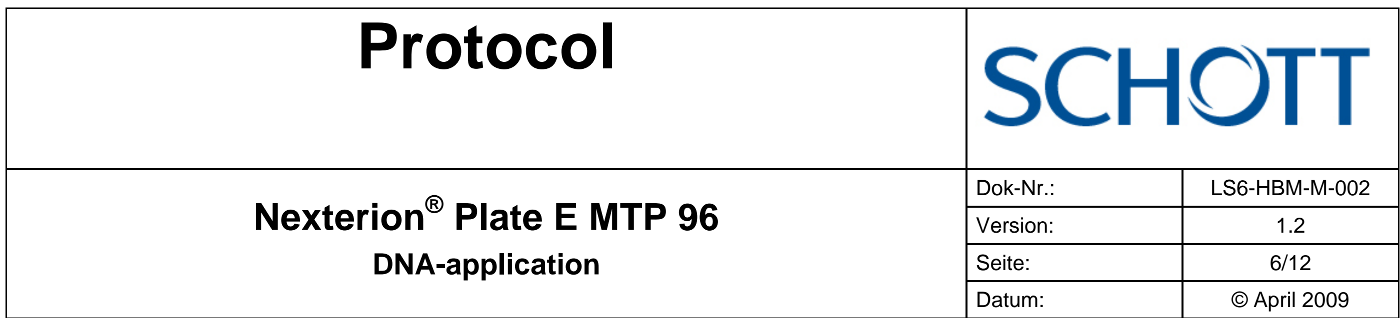
Nexterion® Plate E MTP 96 is compatible with all microarray printing and spotting methods, including contact and non-contact printing technologies.
The following table displays the maximum number of probes per subarray well for Nexterion® Plate E MTP 96 based on theoretical calculations using various 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.schott.com/nexterion

Table 1: Maximum Probe Densities for Nexterion MTP-96

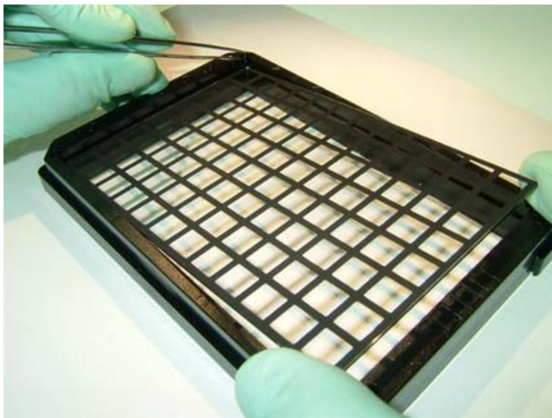
Pitch (µm)	96 Well Sourceplate	384 Well Sourceplate					
Pin Configurations	(1 x 1-2-3-4-6), (2 x 2-2-3-4-6	(1 x 1)	(1 x 2-4-6-8-12)	(2 x 1)	(2 x 2-4-6-8-12)	(4 x 1)	(4 x 2-4-6-8-12)
100	3969	3969	2268	2268	1296	2268	1296
150	1681	1681	902	902	484	902	484
200	961	961	496	496	256	496	256
250	576	576	288	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

Legend	
1 Pin/MTP Well	Buffer is 300 µm + ½ pitch
2 Pins/MTP Well	
4 Pins/MTP Well	



8 DNA immobilization

1. Remove the printed microarray plate (Step 4) from the tray, and incubate printed microarray plates in humidity chamber at room temperature for 30 min (see appendix for details of how to prepare this chamber) to ensure quantitative immobilization.
2. Proceed to washing.



Step 4: Printed plate dissembled for processing.

9 Washing

1. Wash plates to remove unbound probe molecules and buffer substances to avoid interference with subsequent hybridization experiments.
   - Rinse 1 x 5 min in 0.1 % Triton® X-100 at room temperature.
   - Rinse 2 x 2 min in 1 mM HCl solution at room temperature.
   - Rinse 1 x 10 min in 100 mM KCl solution at room temperature.
   - <optional> Denaturation step for arrays spotted with PCR-probes: 1 x 3 min in boiling diH_2O.
   - Rinse 1 x 1 min in diH_2O at room temperature.
2. Proceed to Blocking immediately.

   Note: Make sure that plates do not dry between washing steps and between washing and blocking.

10 Blocking

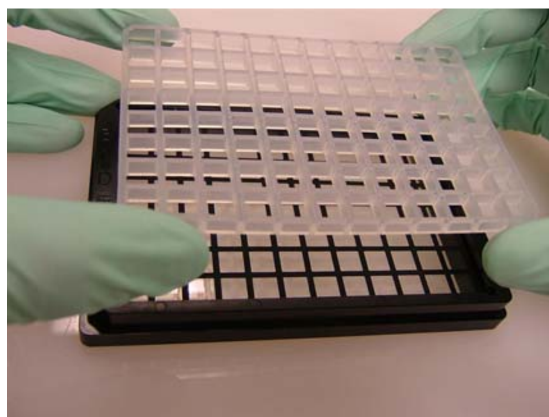
1. Block the plates with Nexterion® Block E Solution or alternatively with 50 mM ethanolamine, 0.1% SDS (add freshly before use) in 0.1 M Tris, pH 9.0 as follows:
   a. Incubate plates 1 x 15 min in 1 x Nexterion® Block E Solution at 50 °C.
   b. Rinse 1 x 1 min in diH_2O at room temperature.
2. Dry the Nexterion® Plate E in an oil-free air or nitrogen stream to avoid any water stains on the plate surface.
3. Proceed to hybridization.

11 Hybridization

1. 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.

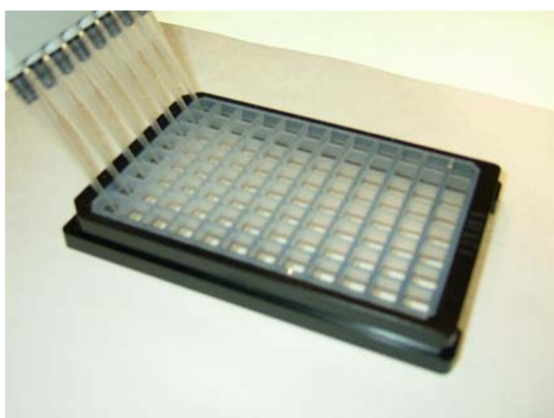
Step 7: Processed microarray plate is ready for hybridization.

2. 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.

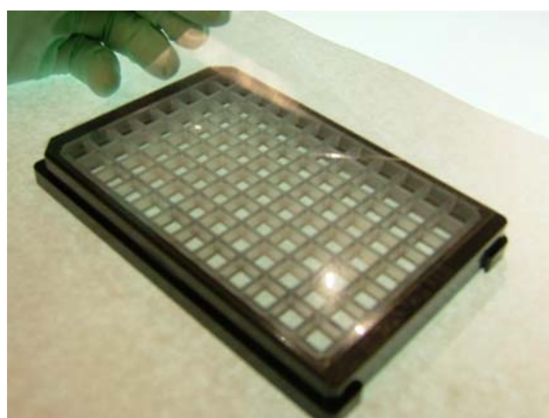
   Note:
   At least 7.5 pmol of each target in 30 µl (maximum volume 200 µl) of hybridization buffer per well is recommended. If the sample is already in solution, then add appropriate amounts of each of the hybridization solution components so that the final concentration for each is the same as listed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

3. 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.

4. Seal the wells immediately with the supplied sealing film (Step 9) ensuring that the seal makes good contact with the superstructure.
5. 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.

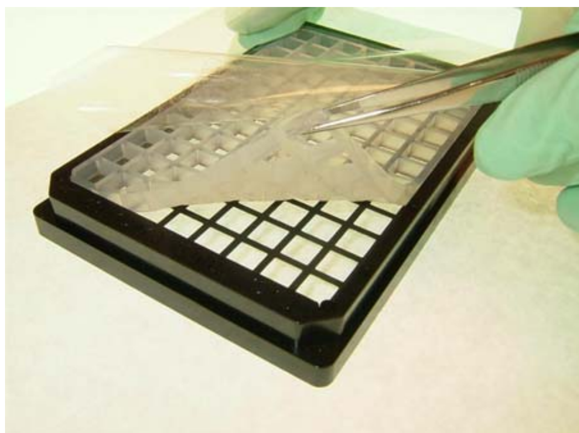
Step 10: Hybridize plate on an orbital shaker.

12 Post-Hybridization washing

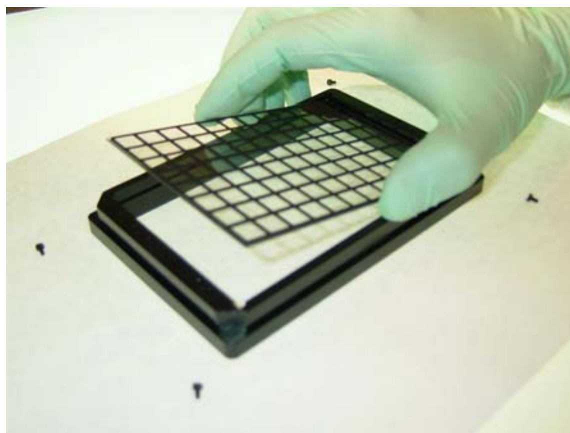
Caution:
Do not allow plates to dry between washes, and protect them from light as much as possible. Never wash the plates with diH_2O after hybridization.

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 2x 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.

13 Appendix

Spotting Probes:
PCR products amplified with amino-modified primers are preferred for spotting. However unmodified PCR products can be also immobilized, because of their exo-cyclic amino-groups.
When an amino-functional primer is used to generate the PCR products, the unused primers should be separated from the PCR products using a suitable method prior to spotting.
Amino-modified short (up to 25 bases) oligonucleotides are immobilized more efficiently than un-modified short oligonucleotides. For longer oligonucleotides the amino-modification does not significantly improve binding efficiency.

Immobilization:
Baking has to be performed in addition to the incubation of printed microarrays at high rel. humidity only for the respective probe type (as indicated) or when using betaine-containing spotting solutions (i.e. 3 x SSC containing 1.5 M betaine).

Unmodified oligonucleotides and unmodified PCR products	All probe types dissolved in a betaine containing spotting solution
Incubate the printed microarray slides at 80 °C for 120 min	Incubate the printed microarray slides at 60 ° to 120 °C for at least 60 min

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

Storage of spotted plates:
There are two recommended ways to store the printed plates:
1. Store the plates directly after spotting and any immobilization steps (i.e. humid chamber treatment and baking, if required).
2. Alternatively store the plates after the washing and blocking steps. Ensure that the plates are stored dry and in the dark, ideally sealed under an inert atmosphere or in a desiccator.
For the long-term storage (a period over several months) it is better to store plates immediately after spotting/immobilization, without any post printing treatment. For short-term storage (i.e. up to 4 days) the plates can be stored with or without carrying out the washing and blocking steps.

Preparation of a humidity chamber:
Prepare a suitable enclosed container (such as an acryl-glass container, plastic food container, or desiccator) by adding a tray to hold the plates and underneath this, a large Petri dish. Fill the Petri dish with steaming hot water 30 minutes prior to use. After 30 min the air will be saturated with water and have cooled to room temperature. The humid chamber is

now ready for use. A new chamber must be prepared for each batch of plates and in advance.
An alternative option is to place a Petri dish containing saturated NaCl solution (with lots of undissolved NaCl) in a suitable sealable container. After achieving equilibration (which will take several hours), the relative humidity will be 75 % at 20 °C. This method works well and has the advantage of only needed to be prepared once. plates may be incubated from 2 hours to over night.

Tips for Hybridization:
The amount of buffer depends on the desired target concentration and the size of the hybridization coverslip used.
As an alternative to the Nexterion® Hyb, a buffer with 3 - 5 x SSC containing 0.1 % SDS with and without formamide can be used.
The length of the hybridization time and temperature depends on the target concentration, sequence, length, etc. and needs to be optimized for each special application (i.e. commonly used conditions are 16 h 42 °C when using formamide containing hybridization buffer and 16 h 65 °C when using formamide free hybridization buffer).
If the sample cannot be applied immediately after denaturation, then place it in a 42 °C water-filled well of a heat block.
Work rapidly when adding the target; and the use of filter pipette tips is recommended

Tips for post-hybridization handling:
Do not allow plates to dry in between washes and protect from light as much as possible. Never wash the plates with diH_2O after hybridization.
The solutions recommended below for washing are a general guideline; your application may require alternative washing stringencies. The volume of the washing solution should be at least 200 ml per plate.

14 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.