Instruction Manual for DNA-Microarrays

3-D Amino Surface

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scienion

greiner bio-one

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I. Introduction

Greiner Bio-One and SCIENION have developed an integrated and complete system for the production and use of microarrays. Their combined expertise in injection moulding, surface modifications, chemistry and molecular biology results in a unique polymer-based microarray platform with outstanding quality and performance.

Greiner Bio-One's HTA™Slides are manufactured under very stringent conditions and controls that guarantee their high quality (clean, flat, planar).

In collaboration with PolyAn a proprietary technology is used to produce a novel, three dimensional surface with excellent specific binding properties: 3-D Amino.

SCIENION Buffer Systems offer optimized solutions for all steps of microarray production and use. *sciSPOT-AM* printing buffer leads to optimal DNA-binding capacity on 3-D Amino surfaces. *sciHYB* hybridization buffer creates the most favourable environment for hybridization between labelled cDNA and spotted amplicons, while minimizing cross-hybridization. *sciPROCESS-AM* and *sciWASH* processing and wash buffers are formulated to reduce background signal and deliver a high level of reproducibility.

The following protocol has been optimized for the generation of DNA-microarrays using SCIENION Buffer Systems together with Greiner Bio-One´s HTA™Slides with 3-D Amino surfaces.

II. Product description

A. Greiner Bio-One HTA™Slides

Product	Slides per case	Cat.-No.
HTA™Slide1, 3-D Amino	5	445 830
HTA™Slide1, 3-D Amino	25	445 835

The HTA™Slide1 has dimensions of a standard microscope slide (25 x 75 x 1 mm). Barcode-labelling is offered upon request.

Product	Slides per case	Cat.-No.
HTA™Slide12, 3-D Amino	5	446 830

The HTA™Slide12 has been designed to increase the throughput of microarrays, e.g. in routine diagnostic applications. The HTA™Slide12 is divided into 12 shallow compartments. Each compartment has a printable area of 6 x 6 mm and a low well rim of only 0.5 mm. Printing devices (either contact or non-contact) can move quickly without significant movement of the z-axis allowing ‘on the fly’-printing. By making it feasible to process 12 different samples simultaneously the HTA™Slide12 increases the speed of analysis and lowers the costs significantly without risking cross-contamination. The slide has the standard format of 25 x 75 x1 mm and can be scanned with all commercially available scanners. Barcode-labelling is offered upon request.

Storage Conditions: Packaged HTA™Slides should be stored in the dark at room temperature (20-27°C) and used prior to expiration date. Once the package has been opened, slides should be used within 1 week if stored at room temperature under inert condition inside a desiccator and protected from light.

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B. SCIENION Buffer Systems

Product	Volume (ml)	Cat.-No.
***sciSPOT-AM*, 2x conc.**	**50**	**445 018**

The SCIENION spotting buffer *sciSPOT-AM* is an advanced buffer system containing a mixture of ionic and polymeric materials. *sciSPOT-AM* buffer has been optimized for spotting PCR-products. Use of *sciSPOT-AM* will increase the quality of microarrays prepared by all contact and non-contact DNA printing technologies.

Users will appreciate the following features:

- Ultra-pure reagents used for buffer formulation
- Provides uniform and highly regular printed features (spots)
- Facilitates uniform DNA immobilization within each spot
- Promotes increased target attachment to the microarray surface
- Enables uniform sample drying
- Reduces or eliminates sample drying in printing pins
- Improves printing consistency
- Expedites data analysis
- Minimizes array to array variation

Product	Volume (ml)	Cat.-No.
***sciPROCESS-AM*, 2x conc.**	**500**	**445 020**

The SCIENION processing buffer *sciPROCESS-AM* has been designed for processing cDNA or oligonucleotide microarrays.

Users will appreciate the following features:

- Ultra-pure reagents used for buffer formulation
- All buffers purified by sterile filtration
- Provided pre-mixed as a 2x concentrate
- Can be used for any cDNA or long oligonucleotide microarray application
- Excellent for gene expression applications

Product	Volume (ml)	Cat.-No.
***sciHYB*, 1x conc.**	**1,5**	**445 016**

The SCIENION hybridization buffer *sciHYB* is an advanced hybridization buffer containing a patent-pending mixture of salts, detergents and buffering components. The use of *sciHYB* hybridization buffer will increase the quality of microarray hybridization reactions involving base pairing interactions between complementary nucleic acid chains.

Users will appreciate the following features:

- Ultra-pure reagents used for buffer formulation
- Increases the signal by accelerating the hybridization kinetics
- Increases the sensitivity by reducing background fluorescence
- Reduces surface tension providing a uniform hybridization layer
- Buffering components stabilize extended reactions
- Arrives pre-mixed and sterile, no preparation required

Product	Volume (ml)	Cat.-No.
***sciWASH I*, 5x conc.**	**500**	**445 011**
***sciWASH II*, 5x conc.**	**500**	**445 012**
***sciWASH III*, 5x conc.**	**500**	**445 013**

The SCIENION wash buffers *sciWASH I, II, III* have been designed for hybridization reactions involving cDNA or long oligonucleotide microarrays.

Users will appreciate the following features:

- Ultra-pure reagents used for buffer formulation
- All buffers purified by sterile filtration
- Provided pre-mixed as a 5x concentrate
- Can be used for any cDNA or long oligonucleotide microarray application
- Excellent for gene expression applications

Storage Conditions: All components of the SCIENION Buffer Systems can be stored at normal laboratory ambient temperature (20-27°C).

Safety Considerations: When working with SCIENION Buffer Systems please follow all generally accepted laboratory safety guidelines. At a minimum, wear appropriate personal protective equipment such as a lab coat, safety glasses, powder-free latex gloves, etc. Follow recommended standard operating procedures for any laboratory equipment used in your experiments.

Product use Limitations, Warranty, Disclaimer: SCIENION Buffer Systems have been scientifically developed and are sold **for research purposes only.** SCIENION Buffer Systems are not for use in human diagnostics or for drug purposes. Extreme care and exact attention should be practiced in the use of the materials described herein. Please refer also to the material safety data sheets. All SCIENION products are subject to extensive quality control and are guaranteed to perform as described when used properly. Any problems with

any SCIENION product should be reported to SCIENION immediately. SCIENION's liability is limited to the replacement of the product, or a full refund. Any misuse of this product is the full responsibility of the user, and SCIENION makes no warranty or guarantee under such circumstances.

C. Sample Pack

SCIENION and Greiner Bio-One offer a sample pack with the following content to test HTA™Slides and buffers:

	Quantity
HTA™Slide1, 3-D Amino	1
HTA™Slide12, 3-Amino	1
sciSPOT-AM, 2x conc.	1.6 ml
sciPROCESS-AM, 2x conc.	42 ml
sciHYB	0.6 ml
sciWASH I, 5x conc.	42 ml
sciWASH II, 5x conc.	42 ml
sciWASH III, 5x conc.	42 ml

III. Materials provided by the researcher

- Slide staining racks and glass staining jar (recommended: Thermo Shandon, Cat.-No.121)
- Shaking incubator
- Distilled water
- Ethanol (technical grade)
- Centrifuge with microplate carriers (to spin-dry slides)
- UV-crosslinker (e.g. Stratalinker, Stratagene)
- Heating block or PCR-machine
- Microcentrifuge
- Glass cover slips
- Hybridization chamber (recommended: *sciHYBCHAMBER*)
- 2x SSC
- Watchmaker forceps
- Clean room wipes

IV. DNA Array Preparation and Hybridization

A. Printing

1. Resuspend cDNA to a final concentration of 0.20-0.75 µg/µl.

PCR products (cDNAs) should be purified to remove contaminants that may interfere by attachment to the substrate. All DNA samples should be resuspended to the appropriate concentration in distilled H_2O. Users may want to test a range of different concentrations to determine the optimal target concentration for a particular assay, though the values given above work very well for many different applications.

2. Transfer 4 µl of each DNA sample into a 96- or 384-well microplate.

Sample transfer can be performed manually, with a multi-channel pipetting device, or with a liquid-handling system. Most samples of cDNAs pre-exist in a 96-well or 384-well format owing to the fact that many PCR purification schemes use a microtiter plate format.

3. Add 4.0 µl *sciSPOT-AM* to each 4.0 µl DNA sample.

Pipetting can be performed manually, with a multi-channel pipetting device or with a liquid-handling system. Make sure that the transfer volume of *sciSPOT-AM* is 4.0 µl ± 10% for all samples, as small differences in the concentration of buffer components can produce variability in spot diameter on the printed microarrays.

4. Mix the DNA samples thoroughly by pipetting up and down 10 times.

Insufficient mixing of the samples at this step will result in poor sample loading, inefficient printing and poor microarray quality! Sample evaporation can be minimized by sealing the microtiter plates with an adhesive sealing film. Properly sealed plates can be stored for several weeks at 4°C without a significant loss of volume or DNA integrity. Samples can be stored indefinitely at –20°C or –80°C, though samples should be re-mixed after thawing and prior to arraying.

5. Print the DNA samples onto HTA™Slides by placing the 96-well or 384-well plates on a suitable microarraying device.

The optimal printing environment is a temperature of 20°C and a relative humidity of 45-50%. After printing, the substrates should be processed appropriately to allow efficient attachment of the target DNA to the surface.

B. Processing

Note: Prior to use preheat the *sciPROCESS-AM* 2x buffer to 37°C for 10-15 min and dilute with distilled water! We recommend processing 5 slides in a glass staining jar

(Thermo Shandon Cat.-No.121), which requires 400 ml of buffer for each step in the following protocol. If you use an alternative wash vessel, please adjust your volumes accordingly. Due to their low density HTA™Slides will float in aquatic solutions. Therefore, place a piece of metal or something equivalent on top of the slides.

*For **Sample Packs** we recommend to use 50 ml tubes as wash vessels: one slide per tube filled with 40 ml of buffer / water. Use magnetic stir bar or shaker for agitation. Make sure, that the printed surface is well covered with buffer / water.*

1. Allow the substrate to dry at room temperature for 30 min.

The drying step can be carried on the plate of the microarrayer or in slide boxes with the lid slightly open.

2. Bind DNA to the surface by UV cross linking.

The DNA can be covalently linked to the surface by subjecting the printed side of the substrates to ultraviolet light (e.g. Stratagene Strata linker) at 400-600 mJ.

3. Transfer printed HTA™Slides to a wash station containing preheated *sciPROCESS-AM* and incubate the slides at 55°C for 15-20 min with vigorous agitation.

In order to reduce the background level and to wash the unbound DNA from the substrates, preheat *sciPROCESS-AM* buffer to 55°C before starting with processing. During this step the bound DNA will be denatured. No additional denaturing is required. At the end of the 15-20 min incubation, the next step must be performed quickly, making sure that no *sciPROCESS-AM* solution dries on the microarray.

4. Transfer HTA™Slides to a wash station containing preheated distilled H_2O and incubate at 55°C for 5 min with vigorous agitation.

Make sure distilled H_2O is moving vigorously across the microarray surface.

5. Transfer HTA™Slides to a wash station containing fresh distilled H_2O and wash at 20-25°C for 15 min with vigorous agitation.

Make sure distilled H_2O is moving vigorously across the microarray surface.

6. Transfer HTA™Slides to a wash station containing fresh distilled H_2O and wash at 20-25°C for 15 min with vigorous agitation.

7. Transfer HTA™Slides to a wash station containing ethanol (technical grade) and agitate briefly by gently plunging HTA™Slides up and down.

8. Immediately transfer HTA™Slides to a centrifuge and spin dry for 5 min at 900 rpm.

The cDNA microarray is now ready for hybridization.

C. Hybridization

Note: Prior to use preheat the *sciHYB* hybridization buffer to 42°C for 2 min and and mix by inverting the tube several times.

1. Purify the fluorescent probe with an appropriate purification kit. For hybridization resuspend the probe in 1.0 part distilled H_20 and mix with 9.0 parts of pre-warmed *sciHYB* hybridization buffer (42°C).

For example, a fluorescent probe desiccated to dryness must be resuspended by adding 2.0 µl of distilled H_20, followed by 18.0 µl of pre-warmed *sciHYB* hybridization buffer.

2. Denature the hybridization probe by heating at 95°C for 3 min in an adjustable heating block or PCR-machine.

3. Keep the probe at 60°C for 1 min and centrifuge briefly.

4. Transfer the HTA™Slides to a *sciHYBCHAMBER* containing 10 µl of 2x SSC in each well.

5. Apply the probe to the microarray:

For HTA™Slides1: use 1 µl of probe in *sciHYB* per cm^2 glass cover slip.
For HTA™Slides12: use 20 µl of probe in *sciHYB* per well.

Covering the wells of an HTA™Slide12 is not necessary. In order to avoid well-to-well cross-contamination do not use a cover slip and handle HTA™Slides12 and the hybridization chamber carefully once the probe has been applied.

6. Close *sciHYBCHAMBER* by tightening the four screws (cross-over) and hybridize overnight at 42°C.

D. Washing

Note: Prior to use preheat *sciWASH* 5x to 37°C for 10-15 min and dilute with distilled water! We recommend washing 3 slides (HTA™Slide1 or HTA™Slide12) in a glass staining jar (Thermo Shandon Cat. -No.121), which requires 400 ml of buffer for each step in the following protocol. If you use an alternative wash vessel, please adjust your volumes accordingly.

1. After hybridization transfer HTA™Slides to a wash station containing *sciWASH I* and wash 3 min at 20-25°C with vigorous agitation.

Make sure to transfer HTA™Slides quickly, to avoid any increase in background fluorescence. If covers or cover slips are used these should fall off during the first 30 s of this step. If the cover slip does not float free from the microarray surface, gentle pressure with fine forceps can be used to remove the cover slip. When using forceps to remove a cover slip, avoid contacting the hybridized surface directly as scratches can reduce the quality of the data. Cover slips should be removed from the wash station prior to the next use to prevent them from scratching the microarray surface during the wash steps.

2. Transfer HTA™Slides to a second wash station containing *sciWASH II* and wash 3 min at 20-25°C with vigorous agitation.

Make sure *sciWASH II* is moving vigorously across the microarray surface.

3. Transfer HTA™Slides to a third wash station containing *sciWASH III* and wash 3 min at 20-25°C with vigorous agitation.

4. Remove excess buffer by blotting one edge of the microarray on a laboratory wipe.

Excess *sciWASH III* should be allowed to drain off the microarray surface for 10-30 s. Clean room wipes or an equivalent lint-free cloth works best for this step.

5. Spin-dry HTA™Slides by using a centrifuge to remove the remaining *sciWASH III*.

A 5 min spin at 900 rpm in a standard centrifuge works well for this step.

6. Scan the microarray to acquire a fluorescent image.

V. Troubleshooting tips

1. Poor printing quality:

   - Incomplete mixing of DNA samples and *sciSPOT-AM*.
   - Poor printing environment: other humidity than 45-50% (inadequate humidity).

2. Microarray shows high background after processing:

   - Be sure to not let any sample dry on the microarray surface while transferring the slides to wash stations.
   - Pay attention to the proper handling and shelf life of the buffer.
   - Do not skip washing the microarray after the processing.

3. Microarray shows high background after hybridization:

   - Impure cDNAs used for spotting
   - Incorrect hybridization temperature
   - Unincorporated dyes were not sufficiently purified away from the probe (use other probe purification kits).
   - Sample dried on the microarray surface during the hybridization reaction.

4. No signal:

   - Poor PCR amplification or defective cDNA microarrays.

- Probes washed away (use wash buffers with shorter wash time).
- Poor probe labelling due to inefficient sample labelling.

VI. Technical assistance

Please contact us if you have any comments, suggestions, or if you need technical assistance:

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