

Technical Instructions for AMINO microarrays

TABLE OF CONTENTS

I. INTRODUCTION	1
II. GENERAL PRECAUTIONS	1
III. REAGENTS REQUIRED	1
IV. EQUIPMENT REQUIRED	1
V. ARRAY PRINTING	2
VI. DNA IMMOBILIZATION	3
VII. WASHING & BLOCKING	3
VIII. HYBRIDIZATION	4
IX. POST-HYBRIDIZATION WASHING	5
X. SCANNING	5

I. INTRODUCTION

Amino Microarray Slides are especially suited for efficient immobilization of cDNA molecules and PCR products. The slide is coated with a multifunctional aminosilane coating, thus providing high electrostatic attraction for increased DNA binding capacity and a long linker arm for optimal probe accessibility.

In combination with the spotting solution **Qlspot** (2x) from **QInstruments** nucleic acid molecules that are dispensed with the various spotting procedures bind covalently to the glass surface.

II. GENERAL PRECAUTIONS

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.

1. Refer to manufacturer supplied Material Safety and Data Sheets (MSDS) for proper handling and disposal of the slides and all chemicals.
2. QInstruments buffers and solutions are for research use only, not for in vitro diagnostic use.

III. REAGENTS REQUIRED

For the spotting and binding of DNA-probes onto Amino surface coating Slides we recommend the optimized QInstruments spotting solution **Qlspot** (2x). For majority of hybridization applications and wash steps we recommend our hybridization buffer **Qlhyb** and wash buffers **Qlwash I, II & III** (10x).

1. Deionized water (dH₂O) - at least 18.2 Megohms-cm resistance is recommended
2. 2x spotting solution **Qlspot** or alternatively 3x SSC or alternatively 1.5 M betaine in 3x SSC or 50 % DMSO
3. Hybridization buffer **Qlhyb** or alternatively 3-5x SSC + 0.1 % SDS
4. Washing buffer **Qlwash I, II & III** (10x)
or alternatively:
 - a) Saline Sodium Citrate (20x SSC) – Ambion #9673
 - b) Sodium Dodecyl Sulfate (SDS) – Fisher #BP166-500 or alternatively 10 % SDS solution for washing (10 g dodecyl sulfate sodium salt in 100 ml dH₂O, dissolve at room temperature)
5. **Pre-Hybridization Buffer:** 25 ml QInstruments **Qlhyb** + 25 ml dH₂O + 500 mg BSA (*for 5 slides*)
6. **Amino Blocking Solution:** 5 g succinic anhydride + 315 ml n-methylpyrrolidone + 35 ml 0.2 M sodium-borate pH 8 (*add sodium-borate freshly before use*).

IV. EQUIPMENT REQUIRED

1. QInstruments incubation chamber **IHC1** for 12 slides or 3 microtiter plates
2. QInstruments hybridization system **HC4-S** or alternatively heat block - capable of heating to 95°C
3. Heated water bath
4. Centrifuge with slide holders or compressed nitrogen gas for drying slides
5. Coplin jars (VWR #25457-006) or slide dish and rack combo (Fisher #900200) for washing slides

V. ARRAY PRINTING

The probes should be dissolved in water and diluted 1:1 with 2x **Qlspot** spotting solution to yield a 1x spotting solution. Therefore, the DNA-molecules have to be adjusted to the **double** of the final spotting concentration according to the recommended values in the following table. For quantitative validations, e.g. gene expression profiles, the DNA-molecules to be immobilized must always be available in excess.

1. Add detergents to **Qlspot** (2x) spotting solution to improve the performance:

Recommended solutions for different applications	
Characteristics	Add detergents to improve the performance
Create relatively small spots for majority of applications	-----
Good results using the Ring-And-Pin Technology	0.02 % Hexadecyltrimethylammonium bromide (C ₁₉ H ₄₂ BrN) <i>Merck, #814119</i>
Create bigger spots with pin and pipetting spotting systems	0.02 % Triton X-100 <i>Merck, #108603</i>
Alternatively spotting solution for Amino surface coating	Mix equal amounts of oligonucleotide probe or PCR product and 50% DMSO to obtain a minimum final probe concentration of 20 µM for oligonucleotides, or 0.3 mg/ml for PCR products in 25% DMSO. Note: For smaller spot sizes, 3xSSC can be used as a printing buffer.

2. Resuspend cDNAs at 0.20-1.0 µg/µl and oligonucleotides at 20-40 µM in distilled H₂O.
3. Transfer 5.0 µl of each resuspended DNA sample into a 96- or 384-well microtiter plate.
4. Add 5.0 µl per well of **Qlspot** (2x) to obtain a recommended final probe concentration according to the following table:

Recommended final spotting concentrations		
Surface coating	Oligonucleotides	PCR products, cDNA
Amino	10 to 20 µM	0.1 to 0.5 µM (0.2 to 1 µg/µl)

5. Mix the samples thoroughly by pipetting up and down 10 times and centrifuge the plate for 5 min.
6. Setup the arrayer according to the manufacturer's recommendations.
7. Print the DNA samples onto Microarray substrate (optimal environment is 20 °C and 40-50 % relative humidity) then allow the arrays to air-dry for min. 10 min.

Notes:

- The results always depend on your equipment, your probes and your experimental conditions.
- The spot morphology and fluorescence uniformity can be modified by the chemical composition and concentration of the spotting solution. The optimal concentration mainly depends on the spotting technology and always needs to be adjusted to the experimental setup.
- **Qlspot** spotting solution is recommended for majority of applications.
- For Ring-And-Pin systems and for pipetting systems based on the capillary principle we also recommend trying out lower concentrations of **Qlspot** spotting solution.
- When using PCR-products containing amino-functional primers, the primer should be separated prior to spotting (e.g. spin columns, microplates for primer removal).
- Use of the 2x **Qlspot** spotting solution is advantageous especially when spotting oligonucleotides up to a length of 50 bases.
- Alternatively 3X SSC or 3X SSC containing 1.5 M betaine or 50 % DMSO can be used as spotting buffers.
- DNA-probes in **Qlspot** 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 – 80°C for 2 min and avoid any change of concentration by condensation.
- If you use a diamond scribe to mark the boundaries of the array, this produces small glass fragments, which may get trapped under the coverslip and damage parts of the array.

VI. DNA IMMOBILIZATION

For completion of the covalent binding of DNA-probes on the slide surface after spotting it is necessary to process the slides as follows:

1. UV-cross link at 250 mJ
- and** 2. Minimum 12 h incubation at room temperature (e.g. store slides over night)

or alternatively:

1. Rehydrate the printed surface for 2-3 seconds by holding the microarray over a heated water bath (95° C).
Caution: Excessive rehydration may result in coalescence of the spots.
2. Snap-dry the printed array (DNA side up) by placing on a heat block at 85° C for 10 seconds.
3. Expose the array side of the slide to UV light using a UV crosslinker at a setting of 250 - 500 mJ.
4. Proceed immediately to pre-hybridization or store the arrays in a desiccator for no more than 2 weeks.

Proceed to Washing

Notes:

- Please note the details of slide manufacturer. The results always depend on your equipment, your probes and your experimental conditions.
- If you do not have access to a UV crosslinker, then bake the slides at 80°C for two hours (*however, UV crosslinking is highly recommended and results in a significantly higher signal intensity compared to baking*).
- After spotting and immobilization, the arrays can be used immediately or stored under dry and dark conditions at room temperature.
- The washing steps after immobilization should not be carried out until immediately prior to hybridization.

VII. WASHING & BLOCKING

After immobilization it is important to remove unbound DNA molecules and buffer substances from the **AMINO** slides by extensive washing to avoid any interference with subsequent hybridization experiments.

To avoid bleeding of the spots it is important to perform the pre-hybridization 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 either done by prehybridization with BSA (**protocol A, blocking past washing**) or by reaction of the NH₂-groups with succinic anhydride (**protocol B, blocking prior to washing**).

Protocol A (with pre-hybridization):

To get 1x Amino slide pre-hybridization buffer for approximately 5 slides:

Surface coating	
Amino	25 ml QInstruments Qlhyb hybridization buffer + 25 ml dH ₂ O + 500 mg BSA

1. 1 x 10 to 20 sec in 0.1 % SDS at room temperature (*dilute 2.5 ml 10 % SDS + 247.5 ml dH₂O*)
2. 1 x 10 to 20 sec in dH₂O at room temperature
3. (*Denaturing step for arrays spotted with PCR-probes*)
1 x 3 min in boiling dH₂O (95 - 100°C)
4. 1 x 45 min in Amino slide pre-hybridization buffer at 42°C
5. 1 x 10 to 20 sec in dH₂O at room temperature
6. Dry slides immediately in an oil-free air or nitrogen stream or centrifuge (2 min at 150 to 200x g) to avoid any water stains on the slide surface.
7. Proceed to hybridization.

Protocol B (with blocking step):

To get 1x Amino slide blocking solution for approximately 20 slides:	
Surface coating	
Amino	5 g succinic anhydride + 315 ml n-methylpyrrolidone + 35 ml 0.2 M sodium-borate pH 8 (add sodium-borate freshly before use)

- 1 x 15 min in Amino slide blocking solution at room temperature
- 1 x 10 to 20 sec in 0.1 % SDS at room temperature (*dilute 2.5 ml 10 % SDS + 247.5 ml dH₂O*)
- 1 x 10 to 20 sec in dH₂O at room temperature
- (Denaturing step for arrays spotted with PCR-probes)
1 x 3 min in boiling dH₂O (95 - 100°C)
- Dry slides immediately in an oil-free air or nitrogen stream or centrifuge (2 min at 150 to 200x g) to avoid any water stains on the slide surface.
- Proceed to hybridization.

Notes:

- The volume of washing solution should be at least 250 ml for 5 slides.
- Make sure that slides do not dry between washing steps and between washing and blocking.

VIII. HYBRIDIZATION

The hybridization of Microarray Slides can be done with 'cover slips' or 'gene frames' in **QInstruments hybridization chamber HC4** or in other commercially available hybridization chambers.

1. Labeling probe and Purify using Purification Kit.
2. Re-suspend the dried, fluorescent labeled target that will be applied to the array in **Qlhyb** hybridization buffer. In case the target is already dissolved in a different buffer or in water, the sample can also be diluted in **Qlhyb** to get at least 90% (v/v) in the final hybridization solution (mixture ratio sample:buffer = 1:9).

Recommended final sample concentrations	
Sample	
cDNA / Oligonucleotide and other DNA molecules	Dilute sample in Qlhyb to get at least 90% (v/v) in the final hybridization solution, mixture ratio sample:buffer = 1:9

Notes:

- The amount of buffer depends on the desired target concentration and the size of hybridization chamber used.
 - As an alternative to the QInstruments hybridization buffer, a buffer with 3-5x SSC + 0.1 % SDS can be used.
3. Denature the 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 micro-centrifuge.
- Note:**
- If the sample cannot be applied immediately after denaturation, then place it in a 42°C water-filled well of a heat block (e.g. QInstruments **HC4-S**).
4. Pipette the appropriate volume onto the array surface of a blocked slide under the cover slip and inside a **QInstruments hybridization chamber HC4**.
 5. Hybridize the probe to the microarray under the appropriate conditions. QInstruments hybridization chamber **HC4** or temperature systems **HC4-S** or **HC4-S2** are recommended.

IX. POST-HYBRIDIZATION WASHING

Wash away unbound labeled probe. QInstruments microarray wash buffers **QIwash I, II & III** (10x) are recommended.

To get 500 ml of 1x wash buffer:	
Wash buffer	
QIwash I	Dilute 50 ml 10x QIwash I with 450 ml dH ₂ O (<i>mix under agitation</i>)
QIwash II	Dilute 50 ml 10x QIwash II with 450 ml dH ₂ O (<i>mix under agitation</i>)
QIwash III	Dilute 50 ml 10x QIwash III with 450 ml dH ₂ O (<i>mix under agitation</i>)

Cautions:

- Do not allow slides to dry between washes, and protect from light as much as possible.
- Never wash the slides with dH₂O after hybridization.
- The solutions recommended below for washing are a general guideline; your application may require alternative stringency washes.
- Make all wash steps with vigorous agitation.

1. Place the microarray slides (max 10 pcs.) into a slide rack and immerse in a dish containing 500 ml **QIwash I**. Wash in the above solution 1 x 10 min at room temperature.
2. Wash 1 x 10 min in 500 ml **QIwash II** at room temperature.
3. Wash 1 x 10 min in 500 ml **QIwash III** at room temperature.

or alternatively Step 1 to 3:

- a) Place the array into a slide rack and immerse in a dish containing 2x SSC and 0.2% SDS. Wash in the above solution 1 x 10 min at room temperature.
- b) Wash 1 x 10 min in 2x SSC.
- c) Wash 1 x 10 min in 0.2x SSC at room temperature.

Notes:

- The volume of the washing solution should be at least 250 ml for 5 Slides.
4. Dry the microarray slides in an oil free air or nitrogen stream or by centrifugation at 2 min at 150 to 200x g to avoid water stains on the slide surface.
 5. Protect the array from light, dust and abrasion of the array surface, until ready for scanning.

X. SCANNING

Scan the microarray to acquire a fluorescent image. Ensure that the laser and filter set of the scanner is compatible with the fluorescent labeling of the probe molecules.

For Technical Assistance, please contact:

QUANTIFOIL Instruments GmbH
Löbstedter Str. 101
07749 Jena
Germany

Phone: +49-3641-87612-0
Fax: +49-3641-87612-99
email: info@QInstruments.com

WARRANTY and PATENTS

QUANTIFOIL INSTRUMENTS PRODUCTS HAVE BEEN SCIENTIFICALLY DEVELOPED AND ARE SOLD FOR RESEARCH PURPOSES ONLY. QUANTIFOIL INSTRUMENTS PRODUCTS ARE NOT FOR USE IN HUMAN DIAGNOSTICS OR FOR DRUG PURPOSES, NOR ARE THEY TO BE ADMINISTERED TO HUMANS IN ANY WAY. EXTREME CARE AND EXACT ATTENTION SHOULD BE PRACTICED IN THE USE OF THE MATERIALS DESCRIBED HEREIN.

QUANTIFOIL INSTRUMENTS PRODUCTS ARE SUBJECT TO EXTENSIVE QUALITY CONTROL AND WILL PERFORM AS DESCRIBED WHEN USED PROPERLY. MANUFACTURER'S LIABILITY IS LIMITED TO THE REPLACEMENT OF THE PRODUCT, OR A FULL REFUND EXCEPT FOR WILLFUL CONDUCT OF QUANTIFOIL INSTRUMENTS. ANY MISUSE OF THIS PRODUCT INCLUDING THE USE OF THE WRONG PROTOCOLS IS THE FULL RESPONSIBILITY OF THE USER, AND QUANTIFOIL INSTRUMENTS MAKES NO WARRANTY OR GUARANTEE UNDER SUCH CIRCUMSTANCES.

ALL PRODUCTS ARE INTENDED TO BE USED FOR THE CUSTOMER'S OWN INTERNAL RESEARCH PURPOSES ONLY AND MAY NOT BE USED FOR DRUG DEVELOPMENT OR DIAGNOSTIC PURPOSES, OR FOR HUMAN USE. QUANTIFOIL INSTRUMENTS PRODUCTS AND THE COMPONENTS THEREOF MAY NOT BE RESOLD, MODIFIED FOR RESALE, OR USED IN ANY MANNER IN THE MANUFACTURE OF COMMERCIAL PRODUCTS WITHOUT PRIOR WRITTEN APPROVAL OF QUANTIFOIL INSTRUMENTS. QUANTIFOIL INSTRUMENTS IS IN THE PROCESS OF PATENTING CERTAIN ASPECTS OF THE TECHNOLOGY.

USING ARRAYS BASED ON QUANTIFOIL INSTRUMENTS 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 6,625,225 AND U.S. PATENT NO. 5,830,645. MANUFACTURING AND USE OF PROBE ARRAYS MAY REQUIRE A LICENCE UNDER THE FOLLOWING PATENTS: U.S. PATENT NOS. 6,040,138 OR 5,445,934 OR 5,744,305 AND UNDER THE FOLLOWING PATENTS OWNED BY OXFORD GENE TECHNOLOGY LIMITED („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 QUANTIFOIL INSTRUMENTS PRODUCTS DOES NOT CONVEY ANY LICENCE UNDER ANY OF THE OGT PATENTS OR ANY OF THE OTHER PATENTS REFERRED TO. FOR THE ABOVE MENTIONED OR OTHER APPLICATIONS QUANTIFOIL INSTRUMENTS MAKES 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. TO INQUIRE ABOUT LICENSING UNDER THE OGT PATENTS, PLEASE CONTACT OGT AT licensing@ogt.co.uk