

UltraGAPS™ Coated Slides

Instruction Manual



For Research Use

Cat. No. 40015: UltraGAPS Slides with Bar Code

Cat. No. 40016: UltraGAPS Slides without Bar Code

Cat. No. 40017: UltraGAPS Slides with Bar Code – Bulk Pack

Cat. No. 40018: UltraGAPS Slides without Bar Code – Bulk Pack

Cat. No. 40019: UltraGAPS Slide Starter Kit

For the most current information about these and related products, please visit www.corning.com/lifesciences.

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CONTENTS

Introduction	1
Overview	1
Handling and Care Instructions	2
Storage Instructions	2
Safety Considerations	2
Product Use Limitations, Warranty, Disclaimer	3
Preparation and Hybridization of DNA Microarrays	4
General Considerations	4
Array Fabrication and Stabilization	7
Array Hybridization	8
Preparation of Hybridization Solution	9
Prehybridization	11
Hybridization	12
Post-Hybridization Washes	13
Additional Information	14
Customer Service and Technical Support	14
Corning® Microarray Products	15

INTRODUCTION

Overview

Corning® UltraGAPS™ Coated Slides are the substrate of choice for fabricating DNA microarrays of the highest and most consistent quality. UltraGAPS Coated Slides are recommended for the fabrication of arrays of double-stranded DNA and arrays of oligonucleotides, and when substrate stability and consistency are absolute requirements. UltraGAPS Coated Slides have a uniform, covalently bound coating of pure Gamma Amino Propyl Silane (GAPS). The GAPS coating is applied to both sides of the slides using a proprietary process under tightly controlled manufacturing conditions. UltraGAPS slides offer a printing surface of unmatched cleanliness, high DNA-binding capacity, uniformity, and stability.

Microarray quality is highly dependent on the quality and integrity of the printing substrate. Arrays printed on coated glass of poor quality are likely to produce spots of varying size, shape, and DNA content. The presence of scratches, haze, and contaminating particulates on the slide surface also cause deformation of the arrays as well as high background fluorescence. These problems lead to loss in sensitivity and generally poor results.

UltraGAPS Coated Slides are manufactured under the most stringent conditions to prevent these problems. All slides are cleaned and individually examined for mechanical defects and for the presence of dust and glass particles. GAPS is applied in an environmentally controlled, HEPA-filtered ISO Class 5 facility, resulting in coated slides with highly uniform surface properties and low intrinsic autofluorescence. Surface wettability is consistent across the slide surface to assure uniform spot size and shape and to avoid uncontrolled wicking or poor volume transfer during the printing process. Amine density is also uniform across the slide surface leading to consistent DNA retention across the printed array. The packaging has been developed to ensure compatibility with the GAPS coating and to maintain the appropriate storage environment.

Handling and Care Instructions

To maximize the benefits of using Corning premium substrates, please follow these recommendations:

- ▶ Use the slides in a clean environment. Particles falling onto the slide surface may cause defects in the printed array as well as nuclease contamination. Self-contained printing environments may be required to prevent such contamination.
- ▶ Avoid direct contact with the surface of the slide. Only the print pins and processing solutions should touch the print area to avoid contamination and abrasion of the coating.
- ▶ When using slides without bar codes, clearly mark the side to be printed using a glass-etching tool.
- ▶ If the package of slides has been inadvertently stored at temperatures lower than 20°C, allow it to come to ambient temperature (20 to 25°C) before opening. Otherwise, condensation may form on the slide surface, negatively affecting the uniformity of the coating.
- ▶ Open the pouch just prior to printing. Close the cap on the slide container as soon as possible after removing slides to maintain a closed environment for unused slides. Place the closed container in the pouch to protect the remaining slides and store them in a desiccator. Use the remaining slides within one week of opening the pack.

Storage Instructions

Store UltraGAPS™ slides at ambient temperature in original undamaged packaging, and use slides by the date indicated on the label. Proceed as described in the Handling and Care Instructions after opening the package.

Safety Considerations

When working with the UltraGAPS slides, please follow all generally accepted laboratory safety guidelines. At a minimum, wear the appropriate personal protective equipment such as a lab coat, safety glasses, powder-free gloves, etc. Follow recom-

mended standard operating procedures for any laboratory equipment used in your experiments. Read the Material Safety Data Sheet (MSDS) for appropriate handling of all products. MSDS is available upon request or can be downloaded from www.corning.com/lifesciences.

Product Use Limitations, Warranty, Disclaimer

Corning® UltraGAPS™ Coated Slides are sold for research purposes only and are not intended for resale. This product is not to be used in human diagnostics or for drug purposes, nor is it to be administered to humans in any way. This product contains chemicals that may be harmful if misused. Proper care should be exercised with this product to prevent human contact. Corning products are guaranteed to perform as described when used properly. Manufacturer liability is limited to the replacement of the product or a full refund. Any misuse of this product including failure to follow proper use protocols is the responsibility of the user, and Corning makes no warranty or guarantee under these circumstances.

Certain arrays and/or methods of preparation, analysis or use may be covered by intellectual property rights held by others in certain countries. Use of this product is recommended only for applications for which the user has a license under proprietary rights of third parties or for technology for which a license is not required.

Corning's products may be used in connection with the manufacture, use and/or analysis of oligonucleotide arrays under patents owned by Oxford Gene Technology Limited or related companies ("OGT"), but Corning does not have the right to pass on a licence under any such patents. Therefore, before Corning's products can be used in connection with the manufacture, use, or analysis of oligonucleotide arrays, the user should first check with OGT as to whether a licence is necessary and if so, secure one. To enquire about a licence under OGT's oligonucleotide array patents, please contact licensing@ogt.co.uk. For information about OGT, please visit its website at www.ogt.co.uk.

PREPARATION AND HYBRIDIZATION OF DNA MICROARRAYS

General Considerations

- ▶ *Composition of spotting solution.* The chemical and physical properties of the spotting solution greatly influence DNA retention, spot morphology, and hybridization efficiency. Corning recommends the use of the Pronto![™] Universal Spotting Solution (Cat. No. 40019, 40025, and 40027) because of its low evaporation rate and nuclease-inhibiting properties. Solvent evaporation causes the concentration of DNA and other nonvolatile components of the spotting solution to rise, leading to time-dependent changes in spot quality, suboptimal array uniformity, and the eventual loss of the spotting solution. The most commonly used spotting solution, in order of decreasing physical stability, are: Pronto![™] Universal Spotting Solution, 50% DMSO, 3 x SSC supplemented with 1.5 M betaine, 150 mM NaPO₄, and 3 x SSC. These solutions have successfully been used to fabricate DNA arrays on UltraGAPS[™] slides. DNA dissolved in DMSO-containing media may aggregate if solvent concentration exceeds 70%, which happens upon prolonged use of the source plates and freeze-thaw cycling. Aqueous SSC- and NaPO₄-containing media have a tendency to salt out, which may cause quill pins to clog, and require reconstitution after each print run, which leads to noticeable variability in DNA concentration among source wells.
- ▶ *Preparation of probe DNA.* Double-stranded DNA for spotting on microarrays is generally obtained by amplification of cloned coding sequences. It is important to purify the amplified fragments as the presence of primers and other components of the reaction mix may interfere with binding to the slide and produce background fluorescence upon hybridization. Be sure to use purification methods that do not contribute fluorescent materials. Only oligonucleotides of the highest quality should be used for microarraying. The optimal length of oligonucleotides to be arrayed on UltraGAPS

slides for transcriptional profiling is 70 nucleotides. As the GAPS-coated surface provides free amine groups for ionic attachment of the negatively charged phosphate groups of the DNA backbone, functionalization of the oligonucleotides with an amine or other reactive group is not necessary. Oligonucleotides with and without such modifications bind equally efficiently to the UltraGAPS[™] substrate.

- ▶ *Concentration of probe DNA.* The high reactivity of UltraGAPS slides allows the use of dilute printing solutions. The optimal concentration needs to be determined empirically. When too little DNA is used, the DNA spots will not reach signal saturation levels, thus reducing the dynamic range of the array. Conversely, highly concentrated printing solutions can produce spots with “comet tails” and other forms of localized background. The concentration and purity of the DNA should be checked spectrophotometrically as well as electrophoretically. The recommended concentrations to use as a starting point for further optimization are 0.15 mg/mL when spotting dsDNA (e.g., PCR products, genomic DNA) and 0.50 mg/mL when spotting oligonucleotides.
- ▶ *Printing conditions and pin quality.* Follow the instructions provided by the manufacturer of arraying equipment and printing pins. Printing should be performed under controlled environmental conditions, where temperature and relative humidity can be maintained at about 25°C and 45%, respectively. Pins should be selected according to their diameter and loading capacity, depending on the desired array density and the number of arrays to be printed. For example, quill pins of 50 microns in diameter can produce arrays containing over 50,000 features when used to spot DNA dissolved in highly hygroscopic spotting solution such as 50% DMSO and Pronto![™] Universal Spotting Solution. Pin-contact time and the force with which the pin strikes the slide also affect spot size and morphology. Pins must be individually qualified before use. Pins that are broken or otherwise do not conform to specifications must be replaced. Printing and pin-washing steps should be optimized during a test run in preparation for array fabrication.

- ▶ **Immobilization procedures.** Binding of DNA to the GAPS-coated surface is enhanced by UV cross-linking and/or baking. These procedures work equally well for DNA molecules longer than 300 bp. Smaller DNA molecules and oligonucleotides are best immobilized by UV cross-linking. When baking, care should be taken regarding the cleanliness of the oven. Volatile organics can irreversibly contaminate the surface of the array leading to high backgrounds.
- ▶ **Quality and composition of array-processing reagents.** The accuracy and reproducibility of microarray results are highly dependent on the nature of the reagents used to process the arrays. Corning strongly recommends the use of the Pronto!™ Universal Hybridization Kit (Cat. No. 40026, 40028), which is specifically tuned to the market-leading UltraGAPS™ slides, and is meant to provide instant expertise and a standardized, trouble-free microarraying experience. The Pronto! system includes the Long Oligo/cDNA Hybridization Solution, which, as its name indicates, is formulated for hybridization to arrays of both long oligonucleotides and double-stranded DNA. The reagent formulations and processes described in this manual have historically performed well for arrays made on aminosilane-coated slides, but they significantly differ from those of the Pronto! Reagent System. When deciding which reagents to use, it is also important to consider that reagents made in the laboratory environment generally lack the stringently controlled consistency that characterizes the Pronto! Reagents.
- ▶ **Background fluorescence.** The sensitivity, specificity, and reproducibility of microarray hybridization are negatively affected by background fluorescence. Depending on their age, the storage conditions, and the purity of the biological material and other components of the spotting solution used, DNA microarrays may develop high levels of background fluorescence on and around the printed areas, decreasing the specificity of the hybridization signals. The occurrence of “spotted” fluorescence can be minimized by placing arrays in a Corning® 25 Slide Holder (Cat. No. 40081) and storing them in clean desiccators. This form of background fluores-

cence can be eliminated by processing the arrays with the presoaking reagents included in the Pronto! Universal Hybridization Kit (Cat. No. 40026, 40028). The spurious attachment of labeled DNA to the unprinted area of the slide causes high background that interferes with spot identification during data collection and limits the sensitivity and dynamic range of the array. Deactivating and/or blocking the unused surface of the slide greatly reduces the incidence of this form of background and can be achieved by processing the arrays with the presoaking and prehybridization reagents conveniently included in the Pronto!™ Universal Hybridization Kit.

Array Fabrication and Stabilization

1. Prepare source plates (sterile, nuclease-free 384-well storage plates are recommended; Cat. Nos. 3656 and 3672) by dissolving purified probe DNA in the spotting solution.
 - ▶ For *double-stranded DNA*, prepare solutions containing between 0.10 and 0.20 mg/mL.
 - ▶ For *oligonucleotides*, prepare solutions containing between 0.35 and 0.70 mg/mL (the molarity of a 0.5-mg/mL solution of unmodified 70-mers is 22 μM).
2. Set up arrayer and print slides according to manufacturer's or laboratory protocol. Always handle slides by the corners and wear powder-free gloves.
3. Remove arrays from printing platform and place them in original slide container or Corning® 25 Slide Holder (Cat. No. 40081).
4. Incubate arrays in desiccator for 24 to 48 hours (vacuum desiccator works best).
5. (Optional; see note) Rehydrate spotted DNA by holding slide (array side down) over a bath of hot purified water (95 to 100°C) for approximately 5 seconds until condensation of the water vapor is observed across the slide. Snap dry array by placing it (array side up) on a hot plate for 2 seconds.

6. Immobilize spotted DNA

- ▶ For *double-stranded DNA*, use a UV cross-linker to apply 150 to 300 mJ of UV energy. Alternatively, bake the arrays at 80°C for 2 to 4 hours. If baking, place arrays in lidded glass container and make sure oven is clean and free of volatile organics.
- ▶ For *oligonucleotides*, use a UV cross-linker to apply 600 mJ of UV energy. Baking does not work well for oligonucleotide arrays.

7. Place arrays back in storage container and store in desiccator at ambient temperature. Arrays can be stored for up to 6 months prior to hybridization. Exchanging the regular atmospheric air for clean nitrogen gas helps prevent oxidation of spotted material and extends the shelf life of the arrays.

Note: Rehydration and snap drying have historically been done to denature double-stranded DNA spotted in a non-denaturing medium and to evenly distribute the probe DNA within the spotted area. The efficacy of this treatment has not been conclusively demonstrated. If performing this step, utmost care must be taken not to overexpose the array to the hot plate, since doing so will affect the integrity of the spotted DNA and increase background. Do not place arrays in boiling water as this may result in a significant loss of probe DNA and delamination of the coating.

Array Hybridization

Most microarray experiments are designed to measure relative transcript abundance (transcriptional profiling), for which it is necessary to convert RNA into fluorescently labeled cDNA. This instruction manual describes labeling parameters and hybridization protocols related to this application. Other applications for which DNA microarrays made on UltraGAPS™ slides are also used include comparative genomic hybridization, SNP detection, and the study of promoter identity and function. These applications may involve the labeling of other types of nucleic acids, such as genomic DNA and short

oligonucleotides, and the use of other enzymes, such as DNA polymerases and terminal transferases. For transcriptional profiling, we recommend the synthesis of cDNA by reverse transcription of total or mRNA in the presence of cyanine-labeled dCTP. We specifically recommend the use of the Pronto!TM *Plus* Systems (Cat. Nos. 40051 to 40056) for RNA isolation, cDNA synthesis, and array hybridization.

Preparation of Hybridization Solution

The quality and purity of the template RNA and the resulting cDNA are critical factors for successful hybridizations. Determine the yield and purity of the template RNA by measuring absorbance at 260 and 280 nm and by gel analysis. Use only RNA showing a 260/280 ratio between 1.7 and 2.1. After synthesis and purification of the cyanine-labeled target cDNA, measure absorbance at 260, 550, and 650 nm. Best hybridization results are obtained with cDNA having a frequency of incorporation (FOI) of at least 20 labeled nucleotides per thousand. Using cDNA of lower FOI reduces the sensitivity of the assay. An FOI greater than 50 is indicative of incomplete removal of unincorporated labeled nucleotides. Determine the yield and label strength of target cDNA using the following formulae:

$$\text{Amount of target cDNA (ng)} = A_{260} \times 37 \times \text{total volume of cDNA } (\mu\text{L})$$

$$\begin{aligned} \text{Labeled nucleotides incorporated (pmoles)} &= \\ \text{for Cy3}^{\text{®}}: & A_{550} \times \text{total volume of cDNA}/0.15 \\ \text{for Cy5:} & A_{650} \times \text{total volume of cDNA}/0.25 \end{aligned}$$

$$\text{FOI} = \frac{\text{Labeled nucleotides incorporated} \times 324.5}{\text{amount of target cDNA}}$$

Note: These equations were generated using the following constants: One A_{260} unit of single-stranded DNA = 37 $\mu\text{g}/\text{mL}$; extinction coefficient of Cy3 = 150,000 $\text{M}^{-1}\text{cm}^{-1}$ at 550 nm; extinction coefficient of Cy5 = 250,000 $\text{M}^{-1}\text{cm}^{-1}$ at 650 nm; average molar mass of dNTP = 324.5.

1. Prepare fresh hybridization solution consisting of:
 - ▶ For *double-stranded DNA*, 35 to 50% formamide, 5 x SSC, 0.1% SDS, and 0.1 mg/mL of a nucleic-acid blocker such as sonicated salmon sperm DNA or calf thymus DNA
 - ▶ For *long oligonucleotides*, 20 to 35% formamide, 5 x SSC, 0.1% SDS, and 0.1 mg/mL of a nucleic-acid blocker such as sonicated salmon sperm DNA or calf thymus DNA
2. Determine the area of the slide to be exposed to the hybridization solution, and calculate the volume of hybridization solution needed for each array. When using Corning® Cover Glass (Cat. No. 2870-22, 2940-244, and 2940-246), apply 2.5 µL of hybridization solution per cm² of surface area. When using M-Series LifterSlip™, apply 3 µL per cm².
3. Calculate the amount of target cDNA needed for each array. The fluorescence strength required to achieve high levels of sensitivity and broad dynamic range depends on the type of RNA used to synthesize the target cDNA:
 - ▶ For Cy®-cDNA made from mRNA, use 0.25 pmoles of incorporated nucleotides per microliter of hybridization solution, per dye. For example, to hybridize an area covered by one Corning 22 x 22 mm cover glass (approximately 5 cm²), dissolve an amount of cDNA containing 3 pmoles of each Cy3- and Cy5-dCTP in 12 µL of hybridization solution.
 - ▶ For Cy-cDNA made from total RNA, use 1.0 pmoles of incorporated nucleotides per microliter of hybridization solution, per dye. For example, to hybridize an area covered by one Corning 22 x 22 mm cover glass (approximately 5 cm²), dissolve an amount of cDNA containing 12 pmoles of each Cy3- and Cy5-dCTP in 12 µL of hybridization solution.
4. Dissolve the appropriate amount of target cDNA in the required volume of hybridization solution.

5. Incubate the cDNA hybridization solution at 95°C for 5 minutes.
6. Briefly centrifuge the cDNA hybridization solution to collect condensation, and allow it cool to room temperature. Do not place the solution on ice, as this will cause precipitation of some of the components. Protect the labeled cDNA from over-exposure to light to minimize photobleaching.

Prehybridization

Prehybridization should be done immediately preceding the application of the target cDNA onto the arrays. This step has the purpose of blocking the unused surface of the slide and removing loosely bound probe DNA. It is recommended that all target cDNAs be characterized prior to the start of prehybridization. The preparation of the hybridization solutions can be completed during the time arrays are being prehybridized.

1. Prepare prehybridization solution consisting of 5 x SSC, 0.1% SDS, and 0.1 mg/mL BSA. The volumes required to process a given number of arrays depends on type of glassware available. Use Coplin jars to simultaneously process up to 5 arrays using only 50 mL of buffer per step.
2. Warm prehybridization solution to 42°C.
3. Immerse arrays in prehybridization solution and incubate at 42°C for 45 to 60 minutes.
4. Transfer prehybridized arrays to 0.1 x SSC and incubate at ambient temperature (22 to 25°C) for 5 minutes.
5. Repeat Step 4.
6. Transfer arrays to purified water and incubate at ambient temperature for 30 seconds.
7. Dry arrays by blowing high-purity nitrogen over the array or by centrifugation at 1,600 rpm, for 2 minutes. Keep arrays in a dust-free environment while completing the preparation of the hybridization solution.

Hybridization

1. Wash the required number of pieces of Corning® Cover Glass (Cat. No. 2870-22, 2940-244, 2940-246; at least 1 piece of cover glass per array should be processed) with nuclease-free water, followed by ethanol. Dry cover glass by blowing high purity compressed nitrogen or allow to air dry in a dust-free environment.
2. Carefully pipette the target cDNA onto the arrayed surface. Avoid touching the array with the pipette tip and creating air bubbles.
 - ▶ When using Corning cover glass, apply the target cDNA in small volumes along the middle of the array. Carefully lower the cover glass onto the array. Avoid trapping air bubbles between the array and the cover glass. Small air bubbles that do form usually dissipate during hybridization. Transfer array-cover glass assembly to Corning Hybridization Chamber (Cat. No. 2551 or 40080).
 - ▶ When using M-Series LifterSlip™, place cover glass over array and carefully pipette hybridization solution so that it is drawn by capillary force into the space between the cover glass and the array. Transfer array-cover glass assembly to Corning Hybridization Chamber II (Cat. No. 40080 only).
3. Assemble the chamber as described in the Corning Microarray Hybridization Chamber Operating Instructions Manual. Keep the chambers right-side up and in a horizontal position at all times to prevent movement of the cover glass relative to the array.
4. Submerge chamber-array assembly in a water bath or place in a hybridization oven kept at 42°C.
5. Hybridize arrays at 42°C for 12 to 16 hours.

Post-Hybridization Washes

It is extremely important not to allow the arrays to dry out between washes, as this will result in high backgrounds. Multiple containers are needed to perform the washes in the most efficient manner. Have all containers and the volumes of washing solutions ready before starting the procedure. Note that steps 2 and 3 both require solutions prewarmed to 42°C.

1. Disassemble the hybridization chambers.
2. Immerse arrays in 2 x SSC, 0.1% SDS at 42°C until the coverslip moves freely away from the slide.
3. Transfer arrays to 2 x SSC, 0.1% SDS at 42°C for 5 minutes.
4. Transfer arrays to 0.1 x SSC, 0.1% SDS at room temperature for 5 minutes.
5. Repeat step 4.
6. Transfer arrays to 0.1 x SSC at room temperature for 1 minute.
7. Repeat Step 6 four times.
8. Rinse arrays in 0.01 x SSC for 10 seconds.
9. Dry arrays by blowing clean compressed nitrogen or by centrifugation at 1,600 x g for 2 minutes.
10. Store arrays in the Corning® 25 Slide Holder (Cat. No. 40081). Protect arrays from overexposure to light until ready to scan.

Note: Arrays spotted on UltraGAPS™ slides can be hybridized at temperatures up to 65°C. The use of hybridization temperatures higher than 42°C, however, calls for changes in the composition of the hybridization and wash solutions described in this manual, such as exclusion of formamide, to properly adjust their stringency to the requirements of the application at hand.

ADDITIONAL INFORMATION

Customer Service and Technical Support

For a detailed troubleshooting guide, answers to frequently asked questions, and additional information about these and other products, please visit www.corning.com/lifesciences. For questions, further clarification about this protocol, and other technical issues and information not covered in this manual, please e-mail clstechserv@corning.com or call 800.492.1110 (+1.978.635.2200 outside Canada and USA).

Corning® Microarray Products

Cat. No.	Product Description	Qty/Pk	Qty/Cs
40024	Pronto!™ Universal Validation Kit	1	1
40025	Pronto! Universal Printing Kit	1	1
40026	Pronto! Universal Hybridization Kit – for 25 arrays	1	1
40027	Pronto! Universal Spotting Solution – 250 mL	1	1
40015	UltraGAPS™ Coated Slides, with Bar Code	5	25
40016	UltraGAPS Coated Slides, without Bar Code	5	25
40017	UltraGAPS Coated Slides, with Bar Code, Bulk Pack	25	25
40018	UltraGAPS Coated Slides, without Bar Code, Bulk Pack	25	25
40019	UltraGAPS Slide Starter Kit with 5 mL Universal Spotting Solution	10	10
2551	Hybridization Chamber	1	5
40080	Hybridization Chamber II	1	5
40001	Hybridization Chamber O-rings	5	5
2870-22	Corning Cover Glass, Square, 22 x 22 mm, No. 1½	1 oz	10 packs
2940-244	Corning Cover Glass, Rectangular, 24 x 40 mm, No. 1½	1 oz	10 packs
2940-246	Corning Cover Glass, Rectangular, 24 x 60 mm, No. 1½	1 oz	10 packs
3357	96 Well V-bottom Polypropylene Microplate	25	100
3656	384 Well Polypropylene Storage Microplate	25	100
3672	384 Well Microarray Printing Plate, Low Volume	10	50
3099	Universal Lid – Rigid Lid for 96 and 384 Well Microplates	25	50
6569	Aluminum Sealing Tape for 384 Well Blocks and Microplates	100	100
6570	Aluminum Sealing Tape for 96 Well Blocks and Microplates	100	100
40081	Corning 25 Slide Holder	10	20

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