Corning® Epoxide Coated Slides

Instruction Manual

For Research Laboratory Use
Cat. No. 40040: Epoxide Coated Slide Starter Kit
Cat. No. 40041: Epoxide Coated Slides with Bar Code
Cat. No. 40042: Epoxide Coated Slides without Bar Code

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

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INTRODUCTION

Overview

Corning® Epoxide Coated Slides are recommended for the fabrication of oligonucleotide arrays for applications including transcriptional profiling and SNP detection, and when substrate stability and consistency are absolute requirements. Epoxide Coated Slides have a uniform coating of a proprietary epoxide chemistry that enables covalent attachment of unmodified and amino-modified oligonucleotides to the glass substrate. The coating is applied on both sides of the slides using a proprietary process under tightly controlled manufacturing conditions. The 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 of sensitivity and generally poor results.

Epoxide Coated Slides are manufactured under the most stringent conditions to prevent these problems. All slides are cleaned and individually examined for mechanical defects and the presence of dust and glass particles. The epoxide surface is applied in an environmentally controlled, HEPA-filtered ISO Class 5 facility, resulting in coated slides with highly uniform surface properties and low 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 print. Packaging has been developed 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 Epoxide Coated Slides at ambient temperature (20° to 25°C) 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 Epoxide Coated 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 latex gloves, etc. Follow recommended standard operating procedures for any laboratory equipment used in your experiments. Read all Material Safety Data Sheets (MSDS) for appropriate handling of all reagents. MSDS are available upon request or can be downloaded from www.corning.com/lifesciences.

Product Use Limitations, Warranty, Disclaimer

Corning® Epoxide 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 license 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 license is necessary and if so, secure one. To inquire about a license 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 OLIGONUCLEOTIDE ARRAYS

General Considerations

- *Concentration of Probe Oligonucleotides*. The high reactivity of the Epoxide Coated Slides allows the use of dilute spotting solutions. Optimal oligonucleotide concentration for spotting on the Epoxide Coated Slides is between 20 and 50 µM (50 µM is approximately 0.5 mg/mL for 30-mers). When too little DNA is used, the printed 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. Desalted or HPLC purified oligonucleotides may be used. Both amino-modified and unmodified oligonucleotides form covalent bonds with the epoxide groups of the surface of the slides.
- *Arrayer Settings and Pin Quality*. Follow the instructions provided by the manufacturer of arraying equipment and printing pins. Pin contact time and the force with which the pin strikes the slide affect spot size and morphology. Pins must be individually qualified before use. Pins that are either broken or do not conform to specifications can ruin otherwise good arrays. Care must be taken to thoroughly wash the pins between visits to source wells in order to avoid sample carry over. Sonication of the pins for 30 minutes prior to the start of the print run and, in the case of long runs, at one or more points within the run, help keep the pins in good working order.
- *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 Holders (Cat. No. 40081) and storing them in a Microarray Storage Pouch (Cat. No. 40086). This form of background fluorescence can be eliminated by processing the arrays with the presoaking reagents included in the Pronto!™ Universal Hybridization Kits (Cat. Nos. 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 Kits.

Array Fabrication and Stabilization

It is crucially important to fully evaluate the performance of a particular spotting medium under conditions that are as close to working conditions as possible before committing large sets of probes to the formulation. Thorough and properly controlled print tests must be done in order to ensure that the desired spot density and array uniformity is achievable. Once probe DNA is dissolved in a spotting medium, it is very difficult to recover it for reconstitution in a different solvent.

The Pronto! Epoxide Spotting Solution (Cat. No. 40047) is provided ready for use. Dilution of the Epoxide Spotting Solution or addition of other reagents is not necessary. Customers are encouraged to try their own phosphate-buffered spotting media in order to determine which medium produces the best results. Sodium phosphate at a concentration of 150 mM, pH 8.5, has been used very successfully on the Corning Epoxide Coated Slides. Solutions containing DMSO do not work well on epoxide slides.

Please note that it is not necessary to UV crosslink or bake the arrays to achieve covalent attachment of the oligonucleotides.

1. Prepare DNA source plates (sterile, nuclease-free Corning® 384 well polypropylene microplates are recommended, Cat. Nos. 3656 or 3672) by one of either alternative methods a or b. Sufficient volume of printing solution needs to be prepared to cover the bottom of the receiving wells; this corresponds to between 5 to 10 µL per well when using 384 well microplates of standard well volume. Please follow the recommendations of the microarrayer manufacturer.
   a. Dissolve oligonucleotides to a concentration of 20 to 50 µM (see General Considerations for details, page 3) in the spotting solution. Transfer DNA solution to a Corning 384 well microplate.
   b. Alternatively, add the desired volume of spotting solution to wells containing DNA that has been dried by vacuum centrifugation.
2. Set up arrayer and print slides according to the arrayer manufacturer's or laboratory protocol. The printing environment should be free of dust particles, and kept at a temperature of 20° to 22°C, with relative humidity between 55 and 70%.
3. (Optional) Incubate printed arrays at 70 to 75% relative humidity (i.e., in a humidity chamber) kept at ambient temperature (20° to 25°C) for 12 to 17 hours. The printing instrument can also be used for this step if humidity can be controlled. Alternatively, create a humidity chamber by using a saturated salt solution enclosed in an airtight container such as an acrylic or glass desiccator jar. A small glass dish can be used to hold the saturated salt solution in the bottom of the desiccator, and humidity can be monitored with a hygrometer.

   Recommended salt solutions are:

   - Saturated sodium nitrite ($NaNO_2$) will provide ~66% humidity at 20°C
   - Saturated NH_4Cl and KNO_3 will provide ~71.2% humidity at 25°C
   - Saturated NH_4Cl and KNO_3 will provide ~72.6% humidity at 20°C.
4. Place arrays in Corning® 25 Slide Holder (Cat. No. 40081). Place holder containing arrays in Corning Microarray Storage Pouch (Cat. No. 40086), heat-seal pouch, and store in at ambient temperature. Hybridize arrays within 6 months of fabrication. Exchanging the regular atmospheric air for clean nitrogen gas helps prevent oxidation of spotted material and extends the shelf life of the arrays.

Array Hybridization

This instruction manual describes labeling parameters and hybridization protocols for measuring relative transcript abundance (transcriptional profiling), which typically involves the synthesis of Cy®-cDNA by reverse transcription of total RNA or mRNA. Other applications for which DNA microarrays made on Epoxide Coated Slides are also used 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 use of the Pronto!™ *Plus* Systems (Cat. Nos. 40055 and 40056 for direct labeling, and 40075 and 40076 for indirect labeling), which include reagents for RNA isolation, cDNA synthesis, and array hybridization.

Pre-Hybridization

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 solution 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 for 5 minutes.
5. Repeat Step 4 twice, for a total of three washes.
6. Transfer arrays to purified water and incubate at ambient temperature for 30 seconds.
7. Dry arrays by blowing high-purity N_2 over the array or by centrifugation at 1,600 x g for 2 minutes. Keep arrays in a dust-free environment while completing the preparation of the hybridization solution.

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 to 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:

Amount of target cDNA (ng) = A_{260} x 37 x total volume of cDNA (µL)

Labeled nucleotides incorporated (pmoles) =

for Cy®3: A_{550} X total volume of cDNA/0.15

for Cy5: A_{650} X total volume of cDNA/0.25

FOI = Labeled nucleotides incorporated x 324.5/amount of target cDNA

Note: These equations were generated using the following constants: One A_{260} unit of single-stranded DNA = 37 µg/mL; Extinction Coefficient of Cy3 = 150,000 $M^{-1}cm^{-1}$ at 550nm; Extinction Coefficient of Cy5 = 250,000 $M^{-1}cm^{-1}$ at 650 nm; Average Molar Mass of dNTP = 324.5.

1. Prepare fresh hybridization solution consisting of:
   - For short oligonucleotides (~30-mers), 10% 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 (50 to 70-mers), 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. Nos. 2870-22, 2940-244, and 2940-246), apply 2.5 µL of hybridization solution per cm^2 of surface area. When using raised-edge coverslips, apply 3 µL per cm^2.
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 cm^2), 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 cm^2), 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 to 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 overexposure to light to minimize photobleaching.

Hybridization

1. Wash the required number of pieces of cover glass with nuclease-free water, followed by ethanol. Dry cover glass by blowing high-purity compressed N_2 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. Apply the target cDNA in small volumes along the middle of the array. Carefully lower the cover glass onto 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 II (Cat. No. 40080).
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 cover glass 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 1 x SSC at room temperature for 2 minutes.
5. Repeat step 4.
6. Transfer arrays to 0.1 x SSC at room temperature for 1 minute.
7. Repeat Step 6.
8. Dry arrays by blowing clean compressed N_2 or by centrifugation at 1,600 x g for 2 minutes.
9. Store arrays in a Corning® 25 Slide Holders (Cat. No. 40081). Protect arrays from overexposure to light until ready to scan.

Note: Arrays fabricated on Epoxide Coated 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 or adjustment of salt concentrations, to properly adjust their stringency to the requirements of the application at hand.

Customer Service and Technical Support

For a detailed troubleshooting guide, end-user FAQ and additional product information 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 (+1.978.442.2200 outside Canada and USA).

Corning® Microarray Products

Cat. No.	Product Description	Qty/Pk	Qty/Cs
40041	Epoxide Coated Slides with Bar Code	5	25
40042	Epoxide Coated Slides without Bar Code	5	25
40040	Epoxide Coated Slide Starter Kit with 5 mL Epoxide Spotting Solution and 0.8 mL Hybridization Solution	5	10
40047	Pronto!™ Epoxide Spotting Solution – 250 mL	1	1
40028	Pronto! Universal Hybridization Kit for 10 Arrays	1	1
40026	Pronto! Universal Hybridization Kit for 25 Arrays	1	1
40029	Pronto! Background Reduction Kit – for 50 Arrays	1	1
40055	Pronto! *Plus* Direct System – for 25 Reactions with RNA Isolation	1	1
40056	Pronto! *Plus* Direct System – for 25 Reactions without RNA Isolation	1	1
40075	Pronto! *Plus* Indirect System – for 25 Reactions with RNA Isolation	1	1
40076	Pronto! *Plus* Indirect System – for 25 Reactions without RNA Isolation	1	1
40080	Hybridization Chamber II	1	5
40081	Corning 25 Slide Mailer	20	20
40082	Corning 5 Slide Mailer	50	50
40085	Microarray Storage Pouch – for 5 Slides	100	100
40086	Miroarray Storage Pouch – for 25 Slides	100	100
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

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