Pronto!™ Universal Microarray Reagent System

Instruction Manual

Life Sciences

For Research Laboratory Use Only
Cat. No. 40024: Pronto! Universal Validation Kit
Cat. No. 40025: Pronto! Universal Printing Kit
Cat. No. 40026: Pronto! Universal Hybridization Kit

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

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INTRODUCTION

Overview

Corning® UltraGAPS™ slides have a uniform, covalently bound coating of pure Gamma Amino Propyl Silane. The GAPS coating is applied on 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.

The quality and reliability of microarray results is also dependent on the quality and consistency of the reagents used to print and process the arrays. Corning has developed reagents that are specifically tuned to our market-leading UltraGAPS slides. The Pronto!™ Microarray Reagent Systems have been optimized for use with the UltraGAPS slides such that researchers may achieve the highest possible level of performance, standardization, and technical control throughout the microarray processes.

The use of the Pronto! Spotting Solutions with UltraGAPS slides results in enhanced printing quality and hybridization performance. The spotting solutions show low evaporation rates, resulting in greater stability of the biological content and lower print failure rates, relative to other commonly used printing solutions. The Pronto! Hybridization Solutions have been optimized to create the most favorable environment for hybridization between labeled cDNA and spotted amplicons or long oligonucleotides while minimizing the occurrence of cross-hybridization. The Pronto! Wash Solutions have been formulated to reduce background signal and thus achieve the highest sensitivity.

The combined use of Corning UltraGAPS slides and Pronto! reagents not only results in cost savings, greater convenience, and increased productivity, but, most importantly, work to set a new standard for microarray quality and performance.

Quality Controlled Reagents

All reagents have been manufactured using the highest quality water and chemical components. Reagents are manufactured in a clean room environment following strict manufacturing process controls. Once the reagents are prepared, all reagents are filter sterilized directly into chemically compatible plastic bottles utilizing precise fill control processes. The reagents then undergo stringent quality control to ensure lot to lot reproducibility and consistency. Reagents are visually inspected as well as tested for pH, conductivity, and nuclease contamination. Printing buffers are functionally examined for print performance using Corning UltraGAPS™ slides and Syto® 61 staining procedures to inspect printed control nucleic acids for spot quality, size, DNA retention, and hybridization efficiency. All reagents are functionally tested to exceed strict specifications using labeled controls and following the recommended protocols as outlined in the Pronto!™ Reagent System protocols.

Handling and Care Instructions

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

1. 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.
2. Avoid direct contact with the surface of the slide to be printed. Only the print pins and processing solutions should touch the print area to avoid contamination and abrasion of the coating.
3. When using slides without bar codes, always print on the side facing away from the wall of the plastic container. Clearly mark the side to be printed using a glass-etching tool.
4. If the package of slides has been inadvertently stored at temperatures lower than 20°C, allow it to come to room temperature before opening. Otherwise, condensation may form on the slide surface, negatively affecting the uniformity of the coating.
5. Open the pouch just prior to printing. Close the cap on the slide container as soon as possible after removing slides for use 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.
6. Equilibrate reagents to recommended temperatures prior to use.
7. Thoroughly mix all solutions prior to use. If precipitation occurs, incubate at 37°C and mix until precipitate is no longer seen.

8. Read all Material Safety Data Sheets (MSDS) for appropriate handling of all reagents provided in the Pronto!™ Microarray Reagent Kits; MSDS are available upon request or can be downloaded from the Corning web site at **www.corning.com/lifesciences**.

Storage Instructions

All components of the Pronto! Universal Reagent Kits can be conveniently stored at normal laboratory ambient temperatures (20° to 27°C). All kit components have met functional performance criteria after exposure to temperatures between -20° and 45°C.

Store UltraGAPS™ slides at ambient temperature (20° to 27°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 Pronto! Microarray Reagent Kits 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. Read all Material Safety Data Sheets for appropriate handling of all reagents provided in the Pronto! Microarray Reagent Kits. MSDS are available upon request or can be downloaded from the Corning web site at **www.corning.com/lifesciences**.

Product Use Limitations, Warranty, Disclaimer

Corning® Pronto! Microarray Reagent Systems 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.

PREPARATION AND HYBRIDIZATION OF ARRAYS OF LONG OLIGONUCLEOTIDES OR DOUBLE-STRANDED DNA

General Considerations

The surface of UltraGAPS™ slides is highly reactive towards DNA. The key to producing microarrays of high quality is to take advantage of this high reactivity during the printing process while minimizing the spurious attachment of nucleic acids to the unprinted area during subsequent manipulation of the array. The following are some of the most critical factors to consider:

- *Concentration of the 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 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 as well as electrophoretically. We recommend 0.1 mg/mL as a starting point for further optimization when printing dsDNA (e.g., PCR products, genomic DNA) and 0.5 mg/mL when printing oligonucleotides.
- *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. Make sure to optimize the printing and pin-washing steps before using the Pronto!™ Universal Spotting Solution for the first time.
- *Immobilization Procedures.* UV cross-linking and/or baking enhances binding of DNA to the GAPS coated surface. 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.
- *RNA Integrity.* The RNA concentration and purity should be determined by the absorbance at 260/280 nm and gel analysis. The A260/280 ratio should be 1.8 to 2.0. The purity and integrity of the RNA should be confirmed by gel electrophoresis. To check for DNA contamination, an aliquot of RNA may be digested with RNase and run on an agarose gel. The presence of a smear or bands after RNase treatment is indicative of

DNA contamination. DNA contamination will result in low signals and high background after hybridization.

- *Input of Labeled cDNA.* The optimal frequency of incorporation (FOI = # of dye-labeled nucleotides per 1,000 nucleotides) of a probe is between 20 and 50 dye-labeled nucleotides per 1,000 nucleotides. Lower incorporation will affect the sensitivity of the probe. An FOI greater than 50 dye-labeled nucleotides per 1,000 is also sub-optimal due to low hybridization efficiencies believed to be due to steric hindrance from the cyanine dye molecules.
- *Procedures To Reduce Background Levels.* Depending on their age, the purity of the biological material and other reagents used, and the storage conditions, DNA microarrays may develop significant levels of background fluorescence on and around the printed areas. It is important to eliminate "spotted" fluorescence in order to accurately measure basal levels of transcript abundance. The Pronto!™ Universal Pre-Soak treatment followed by Pre-Hybridization, as detailed below, is very efficient at eliminating background fluorescence.

Printing Protocol

The Pronto! Universal Spotting Solution is provided ready for use. Dilution or addition of other reagents is not necessary. The Universal Spotting Solution is an excellent medium for dissolving oligonucleotides as well as dsDNA for printing microarrays. This proprietary formulation has been tested thoroughly on UltraGAPS™ slides and may be used with either solid or quill pins.

For Oligonucleotide Arrays

1. Prepare DNA source plates (sterile, nuclease-free Corning 384 Well Storage Microplates are recommended; Cat. No. 3656) 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 and 10 µL per well when using 384 well plates. Please follow the recommendations of the microarrayer manufacturer.
   a. Dissolve oligonucleotides to a maximum of 1.0 mg/mL (0.5 is a good starting concentrations for further optimization) in Pronto! Universal Spotting Solution. Transfer DNA solution to Corning 384 well plate.
   b. Alternatively, add the desired volume of Pronto! Universal Spotting Solution to wells containing DNA that has been dried by vacuum centrifugation.
3. Set up arrayer and print slides according to the 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 45 and 55%.

4. Place arrays in a desiccator for up to 48 hours (vacuum desiccator works best).
5. Immobilize spotted oligonucleotides by applying 600 mJ of UV energy to the printed surface.
6 Store arrays in a dry environment at normal laboratory temperature (20° to 25°C). The orange colored plastic containers may be used for long-term storage of the arrays. 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.

For Double-stranded-DNA Arrays

1. Prepare DNA source plates (sterile, nuclease-free Corning 384 Well Storage Microplates are recommended; Cat. No. 3656) 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 and 10 µL per well when using 384 well plates. Please follow the recommendations of the microarrayer manufacturer.
   a. Dissolve dsDNA to a maximum of 0.25 mg/mL (0.1 mg/mL is a good starting concentrations for further optimization) in Pronto!™ Universal Spotting Solution. Transfer DNA solution to the Corning® 384 well plate.
   b. Alternatively, add the desired volume of Pronto! Universal Spotting Solution to wells containing DNA that has been dried by vacuum centrifugation.
2. Set up arrayer and print slides according to the 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 45 and 55%.
3. Place arrays in a desiccator for up to 48 hours (vacuum desiccator works best).
4. Immobilize spotted DNA by applying 150 to 300 mJ of UV light to the printed surface of the array, or by baking the array at 80°C for 2 to 4 hours.
5. Store arrays in a dry environment at normal laboratory temperature (20° to 25°C). The orange colored plastic containers may be used for long-term storage of the arrays. 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.

Labeling Protocol

The Pronto!™ Universal Hybridization Solution has been validated using Cy-labeled cDNA synthesized by reverse transcription of mRNA and Total RNA in the presence of Cy-dCTP. We have consistently obtained good yields and Cy-dCTP incorporation rates with the SuperScript™ II system (Invitrogen) and FluoroLink™ Cy3™- and Cy5™-dCTP (Amersham Bioscience) for Cy-cDNA synthesis, and the QIAquick™ PCR columns (QIAGEN) for Cy-cDNA purification of the Cy-cDNA. We, therefore, strongly recommend these reagents. In addition, the Pronto! Universal Hybridization Solution may also be used to dissolve other types of fluorescently labeled nucleic acids for microarray hybridization.

The quality and cleanliness of the starting RNA and the resulting cDNA are critical factors for successful use of the arrays. It is recommended that RNA quality be thoroughly checked before attempting to synthesize cDNA and that the labeled cDNA be purified and quantitated spectrophotometrically. Exposure of solutions containing fluorescent nucleotides to light should be minimized to prevent photo bleaching. Cy-labeled cDNA is stable at 4°C for several weeks.

Recommended cDNA Labeling Protocol for mRNA

1. Keeping all reagents on ice, mix the following in a 1.5 mL microcentrifuge tube.

   1.5 µL mRNA sample (1 µg/µL)
   1.0 µL Random hexamers (3 µg/µL)
   19.5 µL Nuclease-free water
   Total volume: 22 µL

2. Incubate RNA/primer solution at 70°C for 10 minutes.

3. While the RNA/primer solution is incubating at 70°C, prepare labeling mix as follows. Cy3 and Cy5 should be done in separate tubes.

	Cy3	Cy5
5x SuperScript™ II buffer (Invitrogen)	8 µL	8 µL
0.1 M DTT (Invitrogen)	4 µL	4 µL
dNTPs*	2 µL	3 µL
Cy3 dCTP (1 mM)**	1 µL	0 µL
Cy5 dCTP (1 mM)**	0 µL	1 µL
Nuclease-free water	1 µL	0 µL
SuperScript II (Invitrogen, 200U/µL)	2 µL	2 µL
Total	18 µL	18 µL

*dNTP concentration: 0.5 mM dATP, dTTP, and dGTP, 0.25 mM dCTP. Mix 5 µL of 100 mM each dA, dT and dGTP, 2.5 µL of dCTP with 982.5 µL RNase/DNase free water.

**Cy3-dCTP and Cy5-dCTP are purchased from Amersham Bioscience.

4. Place RNA/primer solution on ice.
5. Add labeling mix to each tube, and mix well.
6. Incubate at 23°C (22°C-25°C) for 10 minutes.
7. Incubate at 42°C for 2 hours.

Recommended cDNA Labeling Protocol for Total RNA

1. Keeping all reagents on ice, mix the following in a 1.5 mL microcentrifuge tube.

   5 µL of Total RNA (1.0 µg/µL)
   1 µL Random hexamers (3 µg/µL)
   1 µL (optional) oligo dT (2 µg/µL)
   15 µL Nuclease-free water
   Total volume: 22 µL

2. Incubate RNA/primer solution at 70°C for 10 minutes.
3. While the RNA/primer solution is incubating at 70°C, prepare labeling mix as follows. Cy3™ and Cy5™ should be done in separate tubes.

	Cy3	Cy5
5x SuperScript™ II buffer (Invitrogen)	8 µL	8 µL
0.1 M DTT (Invitrogen)	4 µL	4 µL
dNTPs*	2 µL	3 µL
Cy3 dCTP (1 mM)**	1 µL	0 µL
Cy5 dCTP (1 mM)**	0 µL	1 µL
Nuclease-free water	1 µL	0 µL
SuperScript II (Invitrogen, 200U /µL)	2 µL	2 µL
Total	18 µL	18 µL

*dNTP concentration: 10 mM dATP, dTTP, and dGTP, 1 mM dCTP. Mix 100 µL of 100 mM dGTP, 100 µL of 100 mM dATP, 100 µL of 100 mM of dTTP and 10 µL of 100 mM of dCTP, 690 µL of RNase/DNase-free water.

**Cy3-dCTP and Cy5-dCTP are purchased from Amersham Bioscience.

4. Place RNA/primer solution on ice.
5. Add labeling mix to each tube, and mix well.
6. Incubate at 23°C (22°C-25°C) for 10 minutes.
7. Incubate at 42°C for 2 hours.

RNase Treatment

1. Add the following to the cDNA-synthesis reaction:

   1.0 µL RNase H (Invitrogen, 1-4 U/µL)
   0.25 µL RNase A (USB, 20-30 U/µL)

2. Incubate at 37°C for 15 minutes.

Purification of Cy-labeled cDNA

We recommend ethanol precipitation of the cDNA followed by purification using QIAquick™ PCR purification columns and reagents (QIAGEN), with the modifications outlined below.

1. Ethanol precipitate the labeled cDNA solution and resuspend in 40 µL deionized H_2O.
2. Add 4 µL of 3M Sodium Acetate, pH 5.2.
3. Add 5 volumes of QIAGEN Buffer PB (220 µL) and heat to 95°C for 30 seconds.
4. Mix well.
5. Apply solution to a QIAquick PCR purification column.
6. Spin at 13,500 rcf for 60 seconds.
7. Discard flow-through.
8. Wash column with 500 µL of Buffer PE, and centrifuge at 13,500 rcf for 60 seconds.
9. Repeat wash three times.
10. Discard flow-through and place the QIAquick column back in the same tube.
11. Centrifuge the column at 13,500 rcf for 60 seconds to remove traces of Buffer PE.
12. Place QIAquick column in a clean 1.5 mL microcentrifuge tube.
13. To elute labeled cDNA, add 30 µL of 0.5X Buffer EB (EB solution diluted with water) to the center of the QIAquick membrane, let the column stand for 1 minute, then centrifuge at 13,500 rcf for 60 seconds.
14. Perform step 13 with an additional 30 µL of 0.5X Buffer EB.
15. Quantitate absorbance at 260, 550, and 650 nm, and calculate frequency of incorporation.

Hybridization Protocol

The volume of Pronto!™ Universal Hybridization Solution needed depends on the size of the printed area and cover glass. For regular cover glass, such as the recommended Corning 2870 and 2940 cover glass product lines, use 3.0-4.0 µL of hybridization solution per cm^2 of surface area. This range of volume will accommodate differences in humidity conditions and hybridization times. The fluorescence strength required to achieve high levels of sensitivity and broad dynamic range depends on the nature of the template used to synthesize the Cy-cDNA:

- *mRNA*. 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 mRNA-derived cDNA containing 3.75-5.0 pmoles of each Cy3™- and Cy5™-dCTP in 15-20 µL of Pronto!™ Universal Hybridization Solution, respectively.
- *Total RNA*. 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 Total-RNA-derived cDNA containing 15-20 pmoles of each Cy3- and Cy5-dCTP in 15-20 µL of Pronto! Universal Hybridization Solution, respectively.

Best results are obtained with cDNAs having a frequency of incorporation (FOI) of 20 to 50 labeled nucleotides per 1,000 nucleotides of cDNA. The FOI can be calculated using the following formulae:

Amount of labeled cDNA (ng) = A_{260} x 37 x total volume (µL)
pmol of dye incorporated =
for Cy3: A_{550} x total volume/0.15
for Cy5: A_{650} x total volume/0.25
FOI = pmol of dye incorporated x 324.5/ng of cDNA

Wash Solution Preparation

The volumes of Wash Reagents A and B provided in the Pronto! Universal Validation and Hybridization Kits are sufficient for processing 10 and 25 arrays, respectively. It is recommended that preparation of wash solutions be performed at one time as described in order to control variation in the preparation. The following volumes for wash solution preparation are for **10 microarrays** and should be adjusted by multiplying by 2.5 for the Hybridization Kit. Carefully follow the order of addition.

Wash Solution 1:	447.5 mL of deionized water (18 MegaOhm Milli-Q™ preferred) 50 mL of Universal Wash Reagent A 2.5 mL of Universal Wash Reagent B
Wash Solution 2:	1425 mL of deionized water (18 MegaOhm Milli-Q preferred) 75 mL of Universal Wash Reagent A
Wash Solution 3:	300 mL of Wash Solution 2 1200 mL of deionized water (18 MegaOhm Milli-Q preferred)

Pre-Soak and Pre-Hybridization

Note: We recommend processing 5 slides in a TPX staining jar (VWR Int., Cat. No. 25640-907) which requires 100 mL of buffer for each step in the following protocol. If you choose to use an alternative wash vessel, please adjust your volumes accordingly.

1. Heat required volumes of both Pronto!™ Universal Pre-Soak Solution and Pronto! Universal Pre-Hybridization Solution to 42ºC for 30 minutes.
2. Add 1 Sodium Borohydride Pre-Soak Tablet to 100 mL of 42ºC Universal Pre-Soak Solution. Allow the tablet to dissolve completely.
3. Immerse arrays in solution from step 2 and incubate at 42ºC for 20 minutes.
4. Transfer arrays to Wash Solution 2 and incubate at ambient temperature for 30 seconds.
5. Repeat step 4 twice.
6. Transfer arrays to 42ºC Universal Pre-Hybridization Solution (from step 1) and incubate for 15 minutes.
7. Transfer arrays to Wash Solution 2 and incubate at ambient temperature for 1 minute.
8. Transfer arrays to Wash Solution 3 and incubate at ambient temperature for 30 seconds.
9. Repeat step 8 twice.
10. Transfer arrays to nuclease-free water and incubate at ambient temperature (22°C-25°C) for 30 seconds.
11. Dry arrays by blowing high-purity nitrogen gas over the array or by centrifugation at 2,500 rpm for 2 minutes.
12. Place slides face up on a fresh clean room wiper (Berkshire Surex® 805 Wipers, Cat. No. SR8050808-16).

Hybridization

1. Wash the required number of pieces of cover glass (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 gas or allow to air-dry in a dust-free environment.
2. Dissolve the appropriate amount of fluorescently labeled cDNA in the required volume of Pronto! Universal Hybridization Solution (see recommendations about working volumes and fluorescence strength above).
3. Incubate the cDNA solution at 95°C for 5 minutes.

4. Centrifuge the cDNA at 13,500 rcf for 2 minutes at room temperature to allow it to cool and collect condensate. Do not place the solution on ice, as this will cause precipitation of some of the components.
5. Place array in Corning® Hybridization Chamber (Cat. No. 2551). Pipette the probe onto the surface of the printed side of the slide. Carefully place the cover glass on the array. Avoid trapping air bubbles between the array and the cover glass. Small air bubbles that do form usually dissipate during hybridization. Assemble the chamber as described in its package insert.
6. Incubate the chamber-array assembly at 42°C for 12 to 16 hours, using a water bath or a hybridization oven.

Post-Hybridization Washes

Note: We recommend processing 5 slides in a TPX staining jar (VWR Int., Cat. No. 25640-907) which requires 100 mL of buffer for each step in the following protocol. If you choose to use an alternative wash vessel, please adjust your volumes accordingly.

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.

1. Heat required volume of Wash Solution 1 to 42ºC for 30 minutes (note that steps 3 and 4 both require pre-warmed solutions).
2. Disassemble the hybridization chambers right side up.
3. Immerse arrays in Wash Solution 1 at 42°C until the cover glass moves freely away from the slide.
4. Transfer arrays to Wash Solution 1 at 42°C and incubate for 5 minutes with gentle agitation.
5. Transfer arrays to Wash Solution 2 at ambient temperature and incubate for 10 minutes with gentle agitation.
6. Transfer arrays to Wash Solution 3 at ambient temperature and incubate for 2 minutes with gentle agitation.
7. Repeat step 6 twice.
8. Dry arrays by blowing clean compressed nitrogen gas or by centrifugation at 2,500 rpm for 2 minutes.
9. Store arrays in lightproof container until ready to scan.

TROUBLESHOOTING GUIDE AND CUSTOMER SERVICE INFORMATION

For a detailed troubleshooting guide, end-user FAQ and additional product information, please visit our website at **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 actoncs@acton.corning.com or call 800-492-1110 (978-635-2200 outside Canada and USA).

LIST OF RECOMMENDED LABORATORY SUPPLIES AND REAGENTS

- SuperScript™ II Reverse Transcriptase (Invitrogen, Cat. No. 18064-014)
- QIAquick™ PCR Purification Kit (QIAGEN, Inc., Cat. No. 28104)
- FluoroLink™ Cy3™-dCTP (Amersham Bioscience, Cat. No. PA53021)
- FluoroLink™ Cy5™-dCTP (Amersahm Bioscience, Cat. No. PA55021)
- Nuclease-free water (Invitrogen, Cat. No. 10977-015)
- Ribonuclease A (RNase A; USB, Cat. No. 70194Y)
- Ribonuclease H (RNase H; Invitrogen, Cat. No. 18021-014)
- 100 mM DTT (included in SuperScript kit)
- dNTPs (Invitrogen, Cat. No. 10297-018)
- Oligo$(dT)_{12\text{-}18}$ Primer (Invitrogen, Cat. No. N420-01)
- Random hexamers (Invitrogen, Cat. No. 48190-011)
- Dust-free clean room wipes (Berkshire Surex® 805 Wipers, Cat. No. SR8050808-16)
- 100 mL TPX staining jar (VWR Int., Cat. No. 25460-907)

CORNING PRODUCTS FOR MICROARRAYS

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	1	1
40015	UltraGAPS™ Slides, w/ Bar Code	5	25
40016	UltraGAPS Slides, w/o Bar Code	5	25
40017	UltraGAPS Slides, w/ Bar Code, Bulk Pack	25	25
40018	UltraGAPS Slides, w/o Bar Code, Bulk Pack	25	25
2551	Hybridization Chamber	1	5
40001	Hybridization Chamber O-rings	5	5
2870	Cover Glass, Square, No. 1½	1 oz	10 oz
2940	Cover Glass, Rectangular, No. 1½	1 oz	10 oz
3635	96 Well UV Microplate	25	50
3679	96 Well Half Area UV Microplate	25	50
3675	384 Well UV Microplate	5	25
3735	384 Well Polypropylene PCR Plate	25	25
6551	96 Well Polypropylene PCR Plate	25	25
3357	96 Well V-bottom Polypropylene Microplate	25	100
3090	96 Well Robolid – Rigid Cover w/Attached Silicone Sealing Mat	25	50
3089	384 Well Robolid– Rigid Cover w/Attached Silicone Sealing Mat	25	50
3656	384 Well Polypropylene Storage Microplate	25	100
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

Visit Corning Life Sciences web site to learn about Corning microplates and other laboratory products: **www.corning.com/lifesciences**.

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