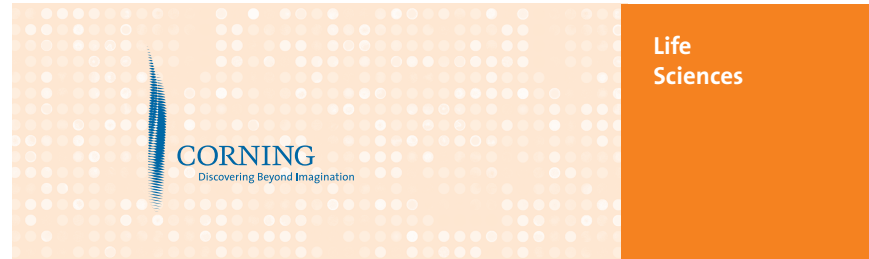


GAPS II Coated Slides

Instruction Manual



For Research Use

Cat. No. 40003: GAPS II Slides with Bar Code

Cat. No. 40005: GAPS II Slides with Bar Code - Bulk Pack

Cat. No. 40004: GAPS II Slides with Bar Code

Cat. No. 40006: GAPS II Slides without Bar Code - Bulk Pack

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Rev. Feb. 2004

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INTRODUCTION

Overview

Corning® GAPS II Coated Slides have a uniform, covalently bound coating of Gamma Amino Propyl Silane (GAPS). The GAPS coating is applied using a proprietary process under tightly controlled manufacturing conditions. GAPS II Coated Slides offer a uniform substrate of unmatched cleanliness and high binding capacity.

Array quality is highly dependent on the quality and integrity of the printing substrate. Arrays printed on coated glass slides of poor quality are likely to produce spots of varying size, shape, and DNA content. Scratches and foreign material on the slide surface also cause deformation of the array as well as varying background fluorescence. These problems lead to loss of sensitivity and generally poor results.

GAPS II 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. The GAPS coating 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 printing process. Amine density is also uniform across the slide surface leading to consistent and highly reproducible retention of macromolecules.

The GAPS II Coated Slides offer a robust, yet economical, substrate to researchers engaged in the fabrication of arrays of double-stranded DNA and arrays of soluble proteins. For more demanding applications, such as the fabrication of oligonucleotide arrays, and when substrate stability and consistency are absolute requirements, we recommend the use of Corning UltraGAPS™ Coated Slides (Cat. Nos. 40015 to 40018).

Handling and Care Instructions

GAPS II Coated Slides are manufactured by a carefully controlled manufacturing process to maximize their performance. To assure this performance, 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 to be printed. Only the print pins and processing solutions should touch the print area to avoid contamination and abrasion of the coating.
- ▶ GAPS II Coated Slides are shipped in 5-slide plastic containers. Two of the five slides in these containers are positioned close to the plastic wall; only the side of these two slides that faces away from the wall should be used for arraying. 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 for use to maintain a closed environment for unused slides. Place the closed container back 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 GAPS II Coated 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 GAPS II 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 recommended standard operating procedures for any laboratory equipment used in your experiments. Read the Material Safety Data Sheet (MSDS) for appropriate handling. MSDS is available upon request or can be downloaded from www.corning.com/lifesciences.

Product Use Limitations, Warranty, Disclaimer

Corning® GAPS II 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 has applied for patents concerning the use of GAPS-coated slides in GPCR membrane microarray applications. GAPS II Coated Slides are not manufactured to the specifications required for use in this application. Purchase of these slides does not imply a license to use GAPS II Coated Slides for GPCR membrane applications.

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 ARRAYS OF DOUBLE-STRANDED DNA

General Considerations

- ▶ *Composition of spotting solution.* The chemical and physical properties of the spotting solution greatly influence DNA retention, spot morphology, and hybridization efficiency. The following spotting solutions have successfully been used to fabricate DNA arrays on GAPS II slides: 15 to 50% DMSO, 3 x SSC, 3 x SSC supplemented with 1.5 M betaine, and 150 mM NaPO₄ (pH 8.5). Solution composed of 50% DMSO is most stable, but produces the largest spots, limiting the achievable feature density. DNA dissolved in DMSO-containing solution 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. Solvent evaporation causes the concentration of DNA and other nonvolatile components of the printing solution to rise, leading to time-dependent changes in spot quality, suboptimal array uniformity, and the eventual loss of the printing solution. The SSC/betaine formulation offers a good balance between physical stability and spot size; however, the removal of betaine

shortly after array fabrication may be necessary to ensure the functionality of the arrays after prolonged storage.

- ▶ *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.
- ▶ *Concentration of probe DNA.* The optimal concentration needs to be determined empirically. The recommended concentration for use as a starting point for further optimization is 0.20 mg/mL. 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.
- ▶ *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. 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 double-stranded DNA molecules longer than 300 bp. Smaller DNA molecules 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.

- ▶ **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 Corning® 25 Slide Holders (Cat. No. 40081) and storing them in clean desiccators. This form of background fluorescence can be eliminated by processing the arrays with the presoaking reagents included in the Pronto![™] Universal Hybridization Kit (Cat. No. 40026). The spurious attachment of labeled DNA to the unprinted area during processing of the array 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 pre-hybridization 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 at a concentration between 0.20 and 0.25 mg/mL.
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 by UV cross-linking or baking. Use a UV cross-linker to apply 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.
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. We specifically recommend the use of the Pronto![™] 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:

Amount of target cDNA (ng) =

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

Labeled nucleotides incorporated (pmoles) =

for Cy3[®]: $A_{550} \times \text{total volume of cDNA} / 0.15$

for Cy5: $A_{650} \times \text{total volume of cDNA} / 0.25$

FOI = Labeled nucleotides incorporated $\times 324.5 /$
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 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.
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 M-Series LifterSlip[™], 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 condensate, 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 overexposure 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 50% formamide, 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 (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 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 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 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 its package insert. 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 Corning® 25 Slide Holder (Cat. No. 40081). Protect arrays from overexposure to light until ready to scan.

Note: Arrays spotted on GAPS II 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.

PREPARATION AND USE OF ARRAYS OF SOLUBLE PROTEINS

The following protocol can be used to array water-soluble polypeptides including histones, DNA-repair proteins, immunoglobulins, and green fluorescence protein, for the study of protein-protein interactions (Coleman MA, Miller KA, Beernink PT, Yoshikawa DM, Albala JS, 2003. Identification of chromatin-related protein interactions using protein microarrays. *Proteomics* 3: 2101-2107). This protocol is recommended as a starting point for work with arrays of soluble proteins and will produce excellent results in most instances. However, given the complexity of the proteome and the wide variety of possible protein interactions, modifications to buffer formulations and detection methods described here may be necessary for certain applications. Some preparations of primary antibodies cross-react with bovine serum albumin (BSA) during the detection process producing high backgrounds. This problem can be avoided by excluding BSA from all buffers.

Array Fabrication and Stabilization

1. Resuspend protein in spotting solution consisting of 50 mM HEPES, pH 7.5, 50 mM KCl, and 5% glycerol, at a final concentration of 0.1 mg/mL. For arraying immunoglobulins, use a spotting solution consisting of phosphate buffered saline (PBS), 0.02% Tween 20, and 1% BSA.
2. Spot proteins on GAPS II slides using a robotic arrayer under regular printing conditions (40% relative humidity, 20 to 25°C).
3. Allow arrays to dry at 25°C in a desiccator for 12 to 24 hours.
5. Store arrays at 4°C until ready to use.

Array Blocking

1. Incubate arrays in blocking buffer consisting of 3% nonfat milk (w/v), 1 x PBS, 1% Tween 20, and 0.1 mg/mL BSA, at 25°C for 15 minutes with gentle shaking.
2. Incubate arrays in wash buffer consisting of 50 mM Tris, pH 7.5, 50 mM NaCl, 2 mM DTT, and 0.5% NP-40, at 25°C for 5 minutes (use this formulation for all washes indicated in this protocol).
3. Repeat Step 2 twice, for a total of 3 washes.

Protein Interaction and Detection

1. Apply ligand-binding buffer consisting of 50 mM sodium phosphate, pH 8.0, 200 mM NaCl, and 5% glycerol, containing 50 to 100 ng of ligand to array.
2. Incubate at 25°C for 30 to 60 minutes with gentle shaking.
3. Wash arrays at 25°C for 5 minutes.
4. Repeat Step 3 twice, for a total of 3 washes.
5. Incubate arrays in a 1:500 dilution of primary antibody in ligand-detection buffer consisting of PBS supplemented with 1% Tween 20. Optimal dilution of primary antibody should be determined empirically.
6. Incubate at 25°C for 30 to 60 minutes with gentle shaking.
7. Wash arrays at 25°C for 5 minutes.
8. Repeat Step 7 twice, for a total of 3 washes.
9. Incubate arrays in a 1:250 dilution of fluorescently labeled secondary antibody in ligand-detection buffer. Optimal dilution of secondary antibody should be determined empirically.
10. Incubate at 25°C for 30 to 60 minutes with gentle shaking.
11. Wash arrays at 25°C for 5 minutes.
12. Repeat Step 11 twice, for a total of 3 washes.
13. Dry arrays by centrifugation at 1,600 x g for 2 minutes.

Array Scanning

Scan arrays at the wavelength corresponding to the emission peak of the fluorophore used. Commonly used fluorescent conjugates include FITC and rhodamine, which have emission peaks at 488 and 543 nm, respectively.

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 40 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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