

DyeSaver2™

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PRODUCT WARNINGS

DANGER! HARMFUL OR FATAL IF SWALLOWED. EXTREMELY FLAMMABLE - VAPORS MAY CAUSE FLASH FIRES! VAPOR HARMFUL. IRRITATES EYES, SKIN AND RESPIRATORY TRACT.

CAUTIONS

CONTAINS KETONES AND TOLUENE.

Contents are **EXTREMELY FLAMMABLE**. Keep away from heat, sparks, and open flame. Use only with adequate ventilation. Avoid contact with eyes and skin. Wash hands after using.

FIRST AID: In case of eye contact, flush thoroughly with large amounts of water for 15 minutes and get medical attention. For skin contact, wash thoroughly with soap and water. In case of respiratory difficulty, provide fresh air and call physician. If swallowed do not induce vomiting. Call Poison Control Center, hospital emergency room, or physician immediately.

DELAYED EFFECTS FROM LONG TERM OVEREXPOSURE. Contains solvents which can cause permanent brain and nervous system damage. Intentional misuse by deliberately concentrating and inhaling the contents can be harmful or fatal.

WARNING: This product contains chemicals known to the State of California to cause cancer and birth defects or other reproductive harm.

**DO NOT TAKE INTERNALLY. KEEP OUT OF THE REACH OF CHILDREN.
FOR PROFESSIONAL USE ONLY. NOT FOR RESIDENTIAL USE.**

DO NOT FREEZE. DO NOT STORE ABOVE 110°F (45°C).

Small, dried spills can be cleaned up with acetone. Please refer to the Material Safety Data Sheet (MSDS) for further information.

OTHER MATERIALS REQUIRED

Materials required for successful use of DyeSaver2 include:

- Glass microarray hybridized with fluorescently labeled probe(s)
- Microarray reader equipped to read Cy3, Cy5, Alexa Fluor 546, Alexa Fluor 647, Alexa Fluor 488, and/or Alexa Fluor 594 fluorochromes
- Clean, blank glass slides
- Glass Coplin jar with screw cap, VWR cat. no. 25457-006 (or equivalent glass container)
- Aluminum foil to line plastic cap of Coplin jar
- Optional: 50mL polypropylene centrifuge tubes
- Optional: Tabletop centrifuge with swinging bucket rotors for 50mL centrifuge tubes
- Optional: 99.8% Pure Toluene, VWR cat. no. BJ347-1
- Optional: 99.9% Pure Acetone, VWR cat. no. BJ010-1

INTRODUCTION

DyeSaver2 is a protective coating that slows the oxidation of fluorescent dyes caused by ozone and other agents commonly present in air. Although similar to the original DyeSaver product, DyeSaver2 offers the following improvements:

- **Better protection:** DyeSaver2 contains three times the protective coating resin as compared to original DyeSaver, which provides a thicker coating that is more resistant to permeation of oxidizing gases after drying.
- **Lower toxicity:** DyeSaver2 contains only 5% toluene as compared to 67% toluene for the original DyeSaver. The most abundant solvents in DyeSaver2 are acetone and ethyl 3-ethoxypropionate, known to be much less toxic than toluene.
- **Lower background:** DyeSaver2 demonstrates very low non-specific fluorescent background signal, particularly in the Cy3 / Alexa Fluor 546 channel, because of its formulation with ultrapure reagents.

Laboratory tests indicate that DyeSaver2 protects Cy3, Cy5, Alexa Fluor 488, Alexa Fluor 594, Alexa Fluor 546, and Alexa Fluor 647 for at least three weeks. DyeSaver2 has been tested for compatibility on glass microarrays with the most common array surfaces (aminosilane, epoxy, and poly-L-lysine) and with probes labeled by direct incorporation, amino allyl, and Genisphere 3DNA kits.

Rapid degradation of Cy5/Alexa Fluor 647 may occur prior to the use of DyeSaver2 from agents in the air or in hybridization and wash buffers. We advise following the recommendations outlined in Appendix B for reducing the degradation of Cy5/Alexa Fluor 647 when performing microarray experiments.

IMPORTANT! DO NOT USE ON VALUABLE OR IRREPLACABLE ARRAYS PRIOR TO DEVELOPING DYESAVER2 APPLICATION TECHNIQUE! We strongly recommend practicing the application of DyeSaver2 on 5 - 10 blank glass slides to ensure even coating and appropriate results. This may be confirmed by scanning the slide before and after application. After appropriate scans are achieved, DyeSaver2 may be applied to hybridized microarrays immediately after the final wash and drying of the array, before scanning.

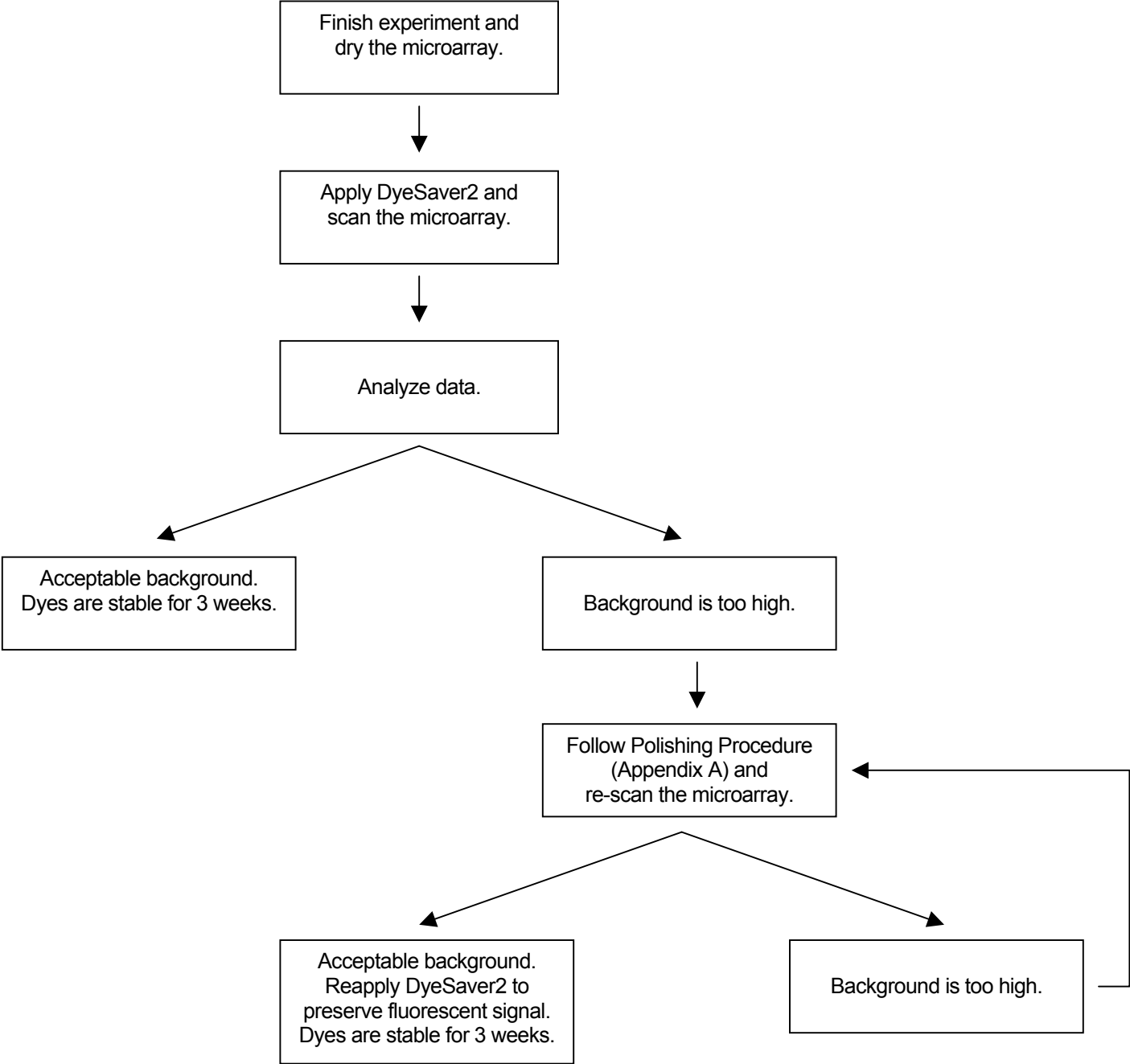
DyeSaver2 will leave a clear appearance on the microarray slide. Use of DyeSaver2 may slightly increase background fluorescence in the Cy3 (532 nm) and Cy5 (635 nm) channels. However, with proper application any increase of background will be uniform and, after background subtraction and normalization, should not adversely affect your results. Background may be further reduced by following the Polishing Procedure in Appendix A. Please note that as DyeSaver2 is removed, the Cy3/Cy5 background will decrease, but protection of Cy3 and Cy5 dyes may also decrease. Therefore, when using the Polishing Procedure to decrease background, the array should be polished immediately before scanning. If necessary, the Polishing Procedure can be repeated until optimal background is achieved, but over-polishing should be avoided because it may result in completely removing the DyeSaver2 coating. After polishing and scanning, DyeSaver2 may be reapplied to better preserve the fluorescent signal (see flow diagram on page 5).

After transferring the DyeSaver2 into a glass Coplin jar, keep the cap of the Coplin jar closed whenever possible. If the jar has a plastic cap, it should be lined with aluminum foil. The solvents of DyeSaver2 will evaporate when the lid is left open. This may lead to the concentration of other components of DyeSaver, which may increase fluorescent background observed on the array. Discard the DyeSaver2 product if you notice a significant increase in fluorescent background due to excess evaporation of DyeSaver or debris in the jar. DyeSaver2 contains organic reagents and should not be used with plastic containers, pipettes, etc.

DyeSaver2 should not be used on oligo arrays from Agilent Technologies.

Questions? Contact Genisphere Technical Support at **877.888.3DNA** or **215.996.9040**.

OVERVIEW OF DYESAVER2 PROCEDURE



PROCEDURE FOR USE

Always handle DyeSaver2 under a chemical fume hood or in a well ventilated area. WEAR GLOVES AND PROTECTIVE EYEWEAR WHEN HANDLING DYESAVER2. Please see precautions and warnings on page 2.

Note: DyeSaver2 application may result in some DyeSaver2 accumulating at the bottom of the slide. This accumulation may be eliminated by following step 6, below.

1. Prepare all materials for use beforehand in a chemical hood or an equivalent well ventilated area. Pour the entire contents of two (2) bottles of DyeSaver2 (56 mL) into a glass Coplin jar (or equivalent glass container). Cap the Coplin jar with a metal screw cap or a plastic screw cap lined with aluminum foil when not in use.
2. After the last wash, dry the microarray by centrifugation or other standard method. Be certain the slide is dust free. If necessary, spray the array with compressed air to remove dust.
3. Keep the slide in a light protective box until applying DyeSaver. Proceed with step 4, below, as quickly as possible to minimize exposure of the slide to oxidizing agents in the air.
4. Holding the labeled end of the slide, dip the array into the Coplin jar with DyeSaver. The array should be submerged for 5-10 seconds. Repeated dipping (up to 2-3 times) of the slide may be required to achieve even coverage with the DyeSaver reagent. Do not submerge any of the slide label or sticker into the DyeSaver.
5. Remove the slide and allow excess liquid DyeSaver to blot onto a paper towel.
6. Optional: Transfer the slide into a 50mL polypropylene centrifuge tube. **DO NOT CAP.** (Do not use polystyrene tubes, as the organic solvents in DyeSaver2 are incompatible with polystyrene). Centrifuge in a table top centrifuge with swinging buckets for 1-3 minutes at 1000-1200 RPM to evenly distribute the DyeSaver over the slide. This will also eliminate any accumulation of DyeSaver at the bottom of the slide. Remove the slide from the centrifuge and discard the 50mL tube. **Caution: Excessive centrifugation will thin the DyeSaver2 coating and reduce its ability to protect against dye fading.**
7. Let the slide dry in an upright (vertical) position for 3-5 minutes.
8. Optional: If necessary, repeat steps 4 through 7 to achieve maximum protection against dye fading. Note that repeat application of DyeSaver2 may cause an increase in fluorescent background. Excess background, thick or uneven coatings of DyeSaver2 may be partially removed ("polished") or completely removed by following Appendix A.
9. Return the slide to a light protective box until ready to scan. The dyes are stable for at least three weeks.

Note: Repeated use of the same glass jar may result in contamination of the DyeSaver2 liquid with dust or debris. These contaminants may adhere to microarrays and produce sub-optimal results. We strongly recommend routine testing of DyeSaver2 by dipping a clean glass slide into the DyeSaver2 solution, and then scanning to determine any presence of debris. If debris is present, discard the DyeSaver2 according to your local hazardous waste disposal regulations.

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Cy is a trademark of Amersham Biosciences.

Alexa Fluor is a trademark of Molecular Probes.

MilliQ is a trademark of Millipore, Inc.

DyeSaver, DyeSaver2, 3DNA, and Genisphere are trademarks of Datascope Corp.

Patents Pending.

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Appendix A

Polishing Procedure

Incomplete surface protection, or non-uniform fluorescent background signal in the Cy3 (532nm) or Cy5 (635nm) channels, may result from an uneven coating of DyeSaver2 on the array surface. If an uneven coating of DyeSaver2 is observed, first determine which side of the slide appears uneven. DyeSaver2 on the back of the slide should not adversely affect the scan of the array. If an uneven coating of DyeSaver2 is on the array side and an unacceptable scan is observed, follow this procedure to either partially remove (“polish”) or completely remove DyeSaver2 from the microarray.

This procedure may also be followed to decrease the background observed on a microarray after DyeSaver2 application. Please note that as DyeSaver2 is removed, the Cy3/Cy5 background will decrease, but protection of Cy3 and Cy5 dyes will also decrease. Therefore, when using the Polishing Procedure to decrease background, the array should be polished immediately before scanning. If necessary, the Polishing Procedure can be repeated until optimal background is achieved, but over-polishing should be avoided because it may result in completely removing the DyeSaver2 coating. **After polishing and scanning, DyeSaver2 may be reapplied to better preserve the fluorescent signal (see flow diagram on page 5).**

1. Prepare a stock Toluene / Acetone solution (3:1, v/v).
2. Transfer 50mL of this stock solvent solution to a glass Coplin jar.
3. Holding the labeled end of the slide, briefly dip the array into the solvent solution. Quickly remove the slide in order to “polish” the DyeSaver application. Repeat **as necessary**. This solvent will remove layers of dried DyeSaver on the slide surface. Additional dips in the solvent solution will eventually remove all of the DyeSaver. Be careful to not submerge any barcode or other label into the solvent solution.
4. Optional: Transfer the slide into a 50mL polypropylene centrifuge tube. DO NOT CAP. (Do not use polystyrene tubes). Centrifuge in a table top centrifuge with swinging buckets for 1-3 minutes at 1000-1200 RPM to remove the polishing solvent. Remove the slide from the centrifuge and discard the 50mL tube.
5. Let the slide dry in an upright (vertical) position.

Appendix B

Recommendations for Reducing the Degradation of Cy5 or Alexa Fluor 647 When Performing Microarray Experiments

Cy5/Alexa Fluor 647 dye performance may be affected by a variety of factors that are particularly prevalent during the summer months. Exposure of the Cy5/Alexa Fluor 647 dye solutions and the hybridized arrays to light and to oxidative environments may cause rapid fading of the Cy5/Alexa Fluor 647 dye, regardless of the labeling system used. Limiting or controlling the exposure of the arrays to these environments has been shown to significantly reduce Cy5/Alexa Fluor 647 fading.

Below are recommendations for reducing the degradation of Cy5/Alexa Fluor 647 when performing microarray experiments:

1. Always keep solutions and arrays containing Cy5/Alexa Fluor 647 away from light, particularly sunlight! Cy5/Alexa Fluor 647 will photobleach when exposed to light, including normal fluorescent lighting!
2. Protect the hybridized, dried array from contact with air, particularly on hot and sunny days. We have found that ambient ozone levels resulting from summertime air pollution can cause oxidative degradation Cy5/Alexa Fluor 647. Keeping the Cy5/Alexa Fluor 647-containing arrays in an inert atmosphere (nitrogen) in a small container (50mL tube) can significantly delay fading of the Cy5/Alexa Fluor 647. Some investigators also add small quantities of dithiothreitol (DTT) or beta mercapto-ethanol (BME) to the bottom of the tube to further promote a reducing micro-environment (be certain to avoid contact of the array with these chemicals).
3. Be careful with the water you use for your post-hybridization wash buffers and other solutions. As noted in the Internet List Serve, MilliQ[®] water has been shown to damage Cy5 (<http://groups.yahoo.com/group/microarray/messages/2867>). Also, be certain that any DEPC treated solutions have had all of the DEPC fully removed (DEPC is a potent oxidizer). Alternatively, we recommend the use of non-DEPC treated nuclease free solutions. Commercially available solutions (water, buffers, etc.) from Ambion have been found to work well with Cy5 labeled microarrays. In addition to Ambion water, we also recommend DI water from VWR (Cat. No. RC91505). Water from Ambion and VWR have been validated for use with microarrays and do not contain components that will oxidize Cy5.
4. Add a small quantity of dithiothreitol (DTT) to the post hybridization wash buffers, i.e. 0.1mM final concentration. This potent reducing agent will protect the Cy5/Alexa Fluor 647 on the array from attack by any oxidative agents in the wash buffers.
5. When using Genisphere 3DNA kits, use the Anti-Fade Reagent in the hybridization solution containing Cy5/Alexa Fluor 647 Capture Reagent. The Anti-Fade Reagent has anti-oxidant properties that will retard the oxidative process.
6. When using Genisphere 3DNA kits, always be certain to mix your 3DNA Cy3 /Alexa Fluor 546 and Cy5/Alexa Fluor 647 Capture Reagents (Vial 1) to break up any aggregates that may form during storage. Follow the heating and vortexing steps outlined in the protocol.