

ProLong[®] Gold and ProLong[®] Diamond Antifade Mountants SlowFade[®] Gold and SlowFade[®] Diamond Antifade Mountants

Table 1 Contents and storage

Material	Amount	Storage*
ProLong [®] Diamond Antifade Mountant	1 × 10 mL (Cat. no. P36970)	<ul style="list-style-type: none"> • 2–8°C • Protect from light
	5 × 2 mL (Cat. no. P36961)	
ProLong [®] Diamond Antifade Mountant with DAPI	1 × 10 mL (Cat. no. P36971)	
	5 × 2 mL (Cat. no. P36962)	
ProLong [®] Gold Antifade Mountant	1 × 10 mL (Cat. no. P36930)	<ul style="list-style-type: none"> • Room temperature (15–30°C) • Protect from light
	5 × 2 mL (Cat. no. P36934)	
ProLong [®] Gold Antifade Mountant with DAPI	1 × 10 mL (Cat. no. P36931)	
	5 × 2 mL (Cat. no. P36935)	
SlowFade [®] Diamond Antifade Mountant	1 × 10 mL (Cat. no. S36972)	<ul style="list-style-type: none"> • 2–8°C • Protect from light
	5 × 2 mL (Cat. no. S36963)	
SlowFade [®] Diamond Antifade Mountant with DAPI	1 × 10 mL (Cat. no. S36973)	
	5 × 2 mL (Cat. no. S36964)	
SlowFade [®] Gold Antifade Mountant	1 × 10 mL (Cat. no. S36936)	<ul style="list-style-type: none"> • Room temperature (15–30°C) • Protect from light
	5 × 2 mL (Cat. no. S36937)	
SlowFade [®] Gold Antifade Mountant with DAPI	1 × 10 mL (Cat. no. S36938)	
	5 × 2 mL (Cat. no. S36939)	

* The product may also be stored at ≤–20°C. When stored as directed, the product is stable for at least 6 months.

Introduction

Life Technologies offers a range of antifade mountants for hard (curing) and soft (non-curing) mount applications in a pre-mixed, ready-to-use format.

The hard mountants, ProLong[®] Diamond and ProLong[®] Gold Antifade Mountant, achieve an optimal refractive index of 1.46 after curing and can be used for long-term storage of slides. *SlowFade*[®] Diamond and *SlowFade*[®] Gold Antifade Mountants are non-curing and the samples can be viewed immediately after mounting with a refractive index of 1.42 at 20°C.

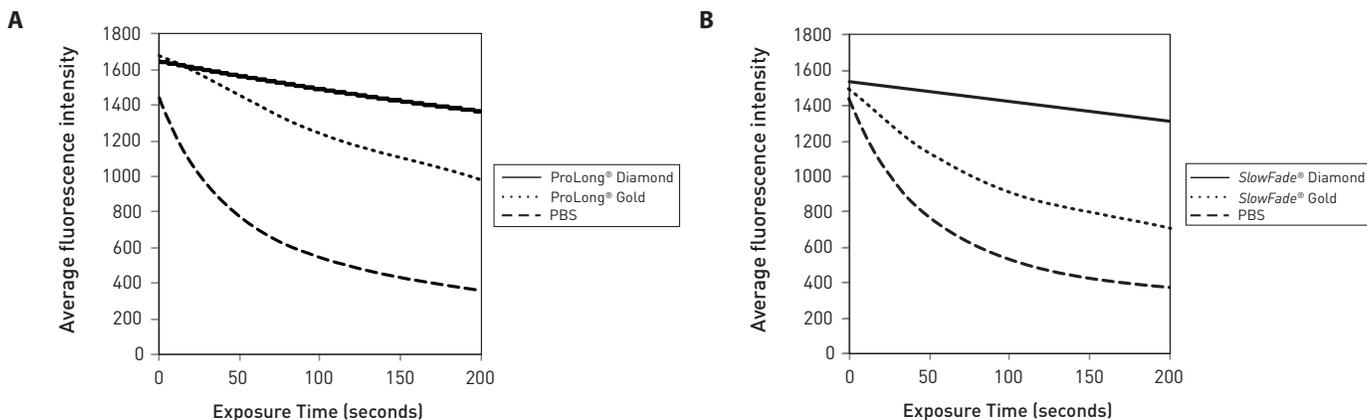
ProLong[®] Diamond Mountant causes little or no quenching of fluorescent signal after mounting and it is the ideal antifade solution for Alexa Fluor[®] dyes, traditional dyes such as FITC and TRITC, and fluorescent proteins such as GFP and mCherry (see Appendix, Table 2, page 6). ProLong[®] Gold Mountant can be conveniently stored at room temperature and provides a similar antifade protection for Alexa Fluor[®] dyes.

SlowFade[®] Diamond Antifade Mountant is a non-curing solution that allows the viewing of samples immediately after mounting. This mountant causes little or no quenching of the fluorescent signal and it is ideal for Alexa Fluor[®] dyes, traditional dyes such as FITC and TRITC, and fluorescent proteins such as GFP and mCherry (see Appendix, Table 2, page 6). *SlowFade*[®] Gold Mountant can be conveniently stored at room temperature and provides similar antifade protection for Alexa Fluor[®] dyes.

When you need to archive the slides for later use, we recommend using the ProLong[®] Diamond or the ProLong[®] Gold Antifade Mountant, which can save the fluorescent signal for weeks or even months, depending upon sample conditions. *SlowFade*[®] Diamond and *SlowFade*[®] Gold Antifade Mountants are intended for short-term storage of slides (2–3 weeks) only; the fluorescence signal from samples mounted using the *SlowFade*[®] mountants may degrade over time.

Both ProLong[®] and *SlowFade*[®] mountants are available with DAPI in the mounting medium to eliminate the need for a separate nuclear counterstaining step.

Figure 1 ProLong[®] Diamond (Panel A) and *SlowFade*[®] Diamond (Panel B) Antifade Mountants provide enhanced resistance to photobleaching. HeLa cells were labeled with a fluorescein (FITC) phalloidin F-actin probe (Life Technologies, Cat. no. F432) and mounted in either PBS or various mounting media. Samples were illuminated for 3 minutes using a 100-watt Hg-arc lamp, imaged using a 20× objective lens, and acquired using a 12-bit monochrome camera. The data plotted is the mean fluorescence intensity from three fields of view over time.



Before You Begin

Important considerations for ProLong® Antifade Mountants

It is important to follow the instructions in this guide when using the ProLong® Gold or the ProLong® Diamond Antifade Mountant. The critical considerations for use of this product are summarized below:

- If needed, warm the bottle of ProLong® Gold or ProLong® Diamond Antifade Mountant to room temperature before use.
- Remove excess moisture from the slide before ProLong® Gold or ProLong® Diamond Antifade Mountant is added by tapping the side of the slide or coverslip on to a clean laboratory wipe.
- Cure the sample after ProLong® Gold or ProLong® Diamond Antifade Mountant is added:
 - Place the mounted sample on a flat, dry surface.
 - Incubate for **24 hours at room temperature in the dark.**

Viewing the sample briefly before curing

To view the sample briefly before curing, tack the corners of the coverslip with epoxy or VALAP. After viewing the sample, allow it to cure for 24 hours at room temperature in the dark on a flat, dry surface.

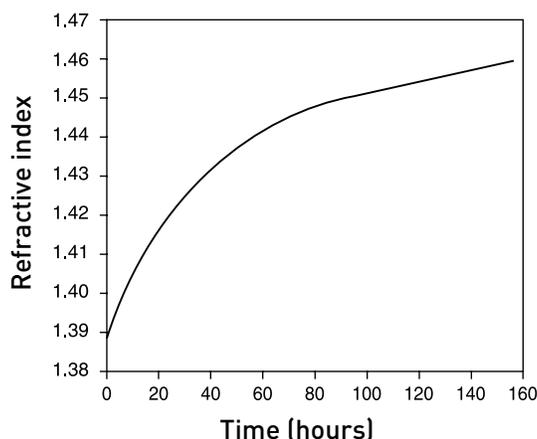
Extended storage of samples

Following the curing time, the edges of the coverslip can be completely sealed with epoxy or VALAP, and the sample stored at room temperature, at 4°C, or at ≤-20°C. Sealing the edges retards the oxidation and extends the life of the sample for several months.

Technical specifications for ProLong® Gold and ProLong® Diamond Antifade Mountants

- pH 7.4 at 20°C
- Refractive index gradually increases as it cures (Figure 2, below)
- ProLong® Gold and ProLong® Diamond Antifade Mountants are useful for long-term storage (many months if edges are sealed), but they must be cured for optimum performance

Figure 2 Increase in the refractive index of ProLong® Gold and ProLong® Diamond Antifade Mountants during the curing process.



Important considerations for *SlowFade*[®] Antifade Mountants

It is important to follow the instructions in this manual when using the *SlowFade*[®] Gold or the *SlowFade*[®] Diamond Antifade Mountant. The critical considerations for use of this product are summarized below:

- If needed, warm the bottle of *SlowFade*[®] Gold or *SlowFade*[®] Diamond Antifade Mountant to room temperature before use.
- Remove excess moisture from the slide by tapping the side of the slide or coverslip on to a clean laboratory wipe before the *SlowFade*[®] Gold or the *SlowFade*[®] Diamond Antifade Mountant is added.

Viewing the sample

Tack the corners of the coverslip with epoxy or VALAP, then image. *SlowFade*[®] Gold and *SlowFade*[®] Diamond Antifade Mountants are intended for short-term use (3–4 weeks); fluorescence signal from samples mounted using this reagent may degrade over time.

Technical specifications for *SlowFade*[®] Gold and *SlowFade*[®] Diamond Antifade Mountants

- pH 7.4 at 20°C
- Refractive index is 1.42 at 20°C

Experimental Protocol

Protocol for mounting samples

These ready-to-use antifade reagents are ideal for use with most fixed samples. Because these solutions contain glycerol, they may be incompatible with some applications, such as mounting specimens that contain lipophilic plasma membrane stains like DiI.

- 1.1 Warm mountant, if needed.** Allow the vial to equilibrate to room temperature. Using an external heat source to warm the vial is not recommended, as this may decrease the long-term stability of the product.
- 1.2 Apply mounting medium.** Remove any excess liquid from the specimen by tapping the side of the slide or coverslip on to a clean laboratory wipe and apply 1 drop (or suitable quantity) of the antifade reagent to the specimen. Cover slide-mounted specimens with a coverslip; for specimens mounted on coverslips, place a drop of antifade reagent onto a clean slide and carefully lower the coverslip onto the antifade reagent to avoid trapping any air bubbles.
- 1.3 Prepare slides for viewing.** For samples mounted using the ProLong[®] Gold or the ProLong[®] Diamond Antifade Mountant, allow the preparation to cure on a flat surface in the dark. Curing time may vary from a couple of hours to overnight, depending on the thickness of the sample and the relative humidity of the surrounding air. We recommend 24 hours curing time.

For long-term storage, seal the coverslip to the slide after curing to prevent excessive shrinkage of the mounting medium, which can result in sample distortion. After sealing, store the slide upright in a covered slide box at room temperature, at 4°C, or at ≤–20°C. Desiccant may be added to the box to ensure that the slide remains dry.

To view the samples immediately, secure the coverslip at the corners using VALAP or hot wax to prevent the coverslip from moving. Leave the edges clear to allow the preparation to cure.

Samples mounted using the *SlowFade*[®] Gold or the *SlowFade*[®] Diamond Antifade Mountant can be imaged immediately after mounting. For thicker samples, it may take time for the antifade reagent to penetrate completely. If necessary, secure the coverslip at the corners using VALAP or hot wax to prevent the coverslip from moving.

Removing mounted coverslip

If you need to remove a mounted coverslip for additional staining, place the mounted slide into a Coplin jar with warm (37°C) phosphate buffered saline (PBS or equivalent physiological buffer), with gentle agitation.

The mountant slowly solubilizes into the buffer and over a period of 30 minutes, the coverslip slides off the slide. If the sample is composed of cultured cells adherent to the coverslip, note the side that the cells are attached after the coverslip comes off. ProLong[®] Gold or ProLong[®] Diamond Antifade Mountant may take longer to dissolve depending on how well it has cured. If using the *SlowFade*[®] Gold or the *SlowFade*[®] Diamond Antifade Mountant, remove any secondary sealing material prior to the process. After removal, wash the sample well with PBS before continuing to remove any residual mounting medium.

Fluorescence microscopy

Samples may be examined with a fluorescence microscope before the mounting medium dries. However, the antifade properties of ProLong[®] Diamond and *SlowFade*[®] Diamond Antifade Mountants do improve slightly the longer they are in contact with the specimen. ProLong[®] Diamond Antifade Mountant in particular achieves maximum effectiveness once it has cured. When properly stored, samples mounted in ProLong[®] Diamond or *SlowFade*[®] Diamond Antifade Mountant continue to resist photobleaching long after they are mounted.

To further reduce photobleaching, minimize the exposure of fluorescently labeled specimens to light by using neutral density filters, and expose samples only when observing or recording a signal. Optimize image capture by using a minimum of optics, high-numerical aperture objectives, relatively low magnification, high-quality optical filters, and high-speed film or high-efficiency detectors.

Appendix

Table 2 Resistance to photobleaching of dyes mounted in ProLong® Diamond, ProLong® Gold, SlowFade® Diamond, and SlowFade® Gold Antifade Mountants.*

Dye	ProLong® Diamond	ProLong® Gold	SlowFade® Diamond	SlowFade® Gold
Alexa Fluor® 488	95%	84%	98%	84%
Alexa Fluor® 546	100%	100%	100%	100%
Alexa Fluor® 555	95%	93%	94%	92%
Alexa Fluor® 568	90%	71%	94%	80%
Alexa Fluor® 594	99%	96%	99%	94%
Alexa Fluor® 647	93%	98%	95%	90%
BODIPY® FL	98%	72%	98%	87%
Cy®3	83%	79%	84%	77%
Cy®5	100%	100%	96%	100%
DAPI	92%	89%	92%	93%
EmGFP	94%	37%	94%	52%
FITC	90%	84%	93%	85%
Hoechst	97%	95%	100%	97%
mCherry	72%	63%	72%	71%
TagRFP	72%	31%	93%	45%
TO-PRO®	87%	90%	98%	78%
TRITC	82%	76%	95%	77%
Texas Red®	92%	93%	96%	92%

*Expressed as percentage of initial fluorescence intensity. Photobleaching resistance was quantified on a Zeiss LSM 710 confocal microscope. HeLa or U2OS cells were stained and mounted using standard immunocytochemistry (ICC) protocols. Five regions within three fields of view were scanned 15× with 1.58 μs dwell time per pixel. Excitation wavelength and intensity were optimized by fluorophore.

Product List

Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
P36961	ProLong [®] Diamond Antifade Mountant	5 × 2 mL
P36970	ProLong [®] Diamond Antifade Mountant	10 mL
P36962	ProLong [®] Diamond Antifade Mountant with DAPI	5 × 2 mL
P36971	ProLong [®] Diamond Antifade Mountant with DAPI	10 mL
S36963	SlowFade [®] Diamond Antifade Mountant	5 × 2 mL
S36972	SlowFade [®] Diamond Antifade Mountant	10 mL
S36964	SlowFade [®] Diamond Antifade Mountant with DAPI	5 × 2 mL
S36973	SlowFade [®] Diamond Antifade Mountant with DAPI	10 mL
P10144	ProLong [®] Gold antifade reagent	2 mL
P36930	ProLong [®] Gold antifade reagent	10 mL
P36934	ProLong [®] Gold antifade reagent *special packaging*	5 × 2 mL
P36941	ProLong [®] Gold antifade reagent with DAPI	2 mL
P36931	ProLong [®] Gold antifade reagent with DAPI	10 mL
P36935	ProLong [®] Gold antifade reagent with DAPI *special packaging*	5 × 2 mL
S36940	SlowFade [®] Gold antifade reagent	2 mL
S36936	SlowFade [®] Gold antifade reagent	10 mL
S36937	SlowFade [®] Gold antifade reagent *special packaging*	5 × 2 mL
S36942	SlowFade [®] Gold antifade reagent with DAPI	2 mL
S36938	SlowFade [®] Gold antifade reagent with DAPI	10 mL
S36939	SlowFade [®] Gold antifade reagent with DAPI *special packaging*	5 × 2 mL

Purchaser Notification

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

Obtaining Support

For the latest services and support information for all locations, go to www.lifetechnologies.com.

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- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
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SDS

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