ProLong® Gold and *SlowFade*® Gold Antifade Reagents

Table 1. Contents and Storage Information.

Material	Amount	Storage	Stability
ProLong® Gold antifade reagent	1 bottle containing 10 mL (P36930)	• ≤–20°C • Protect from light	When stored as directed, the product is stable for at least 6 months
	5 bottles each containing 2 mL (P36934)		
ProLong® Gold antifade reagent with DAPI	1 bottle containing 10 mL (P36931)		
	5 bottles each containing 2 mL (P36935)		
SlowFade® Gold antifade reagent	1 bottle containing 10 mL (S36936)		
	5 bottles each containing 2 mL (S36937)		
SlowFade® Gold antifade reagent with DAPI	1 bottle containing 10 mL (S36938)		
	5 bottles each containing 2 mL (S36939)		

Introduction

Molecular Probes now offers improved versions of the very effective ProLong® and *SlowFade*® antifade reagents. ProLong® Gold antifade reagent (Cat. no. P36930, P36934) offers enhanced resistance to photobleaching and is premixed and ready to use—just add a drop of reagent and mount. ProLong® Gold reagent causes little or no quenching of the fluorescent signal (Figure 1). Like the original ProLong® antifade reagent, ProLong® Gold reagent cures within 24 hours, and the sample can be saved for months after mounting. This antifade reagent offers excellent compatibility with a multitude of dyes and dye complexes (See *Appendix*, Table 2), making it an especially valuable tool for multicolor applications. For convenience, we also offer ProLong® Gold antifade reagent with DAPI (Cat. no. P36931, P36935); the addition of DAPI in the mounting medium eliminates the need for a separate nuclear counterstaining step.

SlowFade® Gold antifade reagent (Cat. no. S36936, S36937) outperforms the original *SlowFade*® reagent, offering increased resistance to photobleaching for a wide range of fluorescent dyes. *SlowFade*® Gold reagent is provided as a premixed and ready-to-use solution. Unlike ProLong® Gold reagent, *SlowFade*® Gold reagent does not cure over time, so samples can be viewed immediately—simply tack the corners of the slide with hot wax or nail polish, then image. *SlowFade*® Gold reagent is intended for short-term use (3–4 weeks) only; samples mounted using *SlowFade*® Gold reagent may degrade over time. *SlowFade*® Gold antifade reagent is also available with DAPI (Cat. no. S36938, S36939).

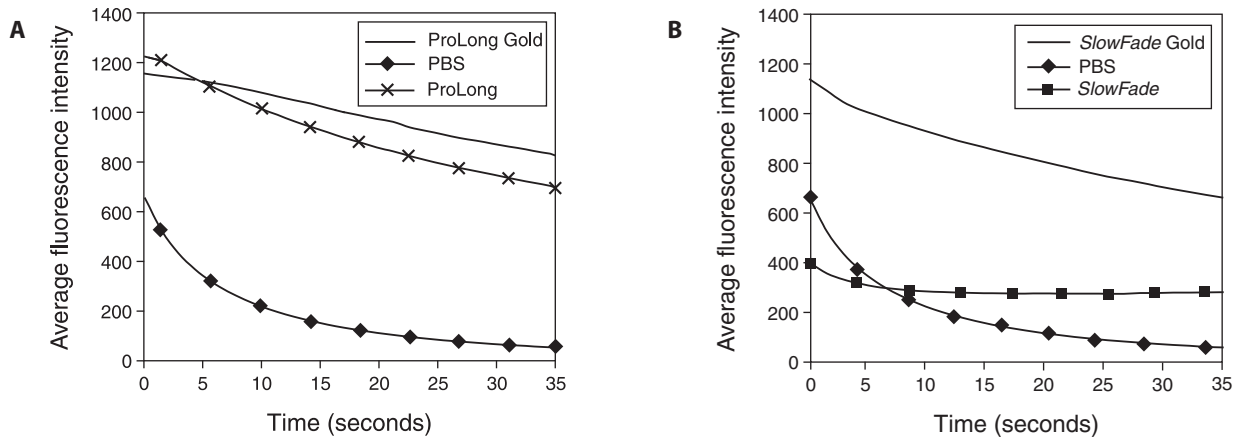

Figure 1. ProLong® Gold (Panel A) and *SlowFade*® Gold (Panel B) antifade reagents provide enhanced resistance to photobleaching. Fluorescein-labeled microspheres were mounted with various antifade reagents and illuminated for 30 seconds using a 100-watt Hg-arc lamp. Samples were imaged using a 40x/1.3 NA oil immersion lens and acquired using a 12-bit monochrome CCD camera. The same exposure settings were used for all images. The data plotted is the average fluorescence intensity from 20 microspheres.

Before You Begin

Important Considerations When Using ProLong® Gold Antifade Reagent

It is important to follow the instructions in this manual when using ProLong® Gold antifade reagent. The critical considerations for use of this product are summarized below:

- Warm the bottle of ProLong® Gold antifade reagent to room temperature before use
- Remove excess moisture from the slide before ProLong® Gold antifade reagent is added by tapping the side of the slide or coverslip on to a clean laboratory wipe
- Cure the sample after ProLong® Gold antifade reagent 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 nail polish. 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 Mounted with ProLong® Gold Antifade Reagent

Following the curing time, the edges of the coverslip can be completely sealed with epoxy or nail polish, 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 Antifade Reagent

- pH 7.4 at 20˚C
- refractive index gradually increases as it cures (Figure 2)
- ProLong® Gold antifade reagent is useful for long-term storage (many months if edges are sealed), but must be cured for optimum performance

Important Considerations When Using *SlowFade*® Gold Antifade Reagent

It is important to follow the instructions in this manual when using *SlowFade*® Gold antifade reagent. The critical considerations for use of this product are summarized below:

- Warm the bottle of *SlowFade*® Gold antifade reagent 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 *SlowFade*® Gold antifade reagent is added

Viewing the Sample

Tack the corners of the coverslip with epoxy or nail polish, then image. *SlowFade*® Gold antifade reagent is intended for short-term use (3–4 weeks); samples mounted using this reagent may degrade over time.

Technical Specifications for *SlowFade*® Gold Antifade Reagent

- 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 reagent. Remove the antifade reagent from the freezer and 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 ProLong® Gold reagent, 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 nail polish or hot wax to prevent the coverslip from moving. Leave the edges clear to allow the preparation to cure. After curing is complete, fully seal the edges for optimum sample longevity.

Samples mounted using *SlowFade*® Gold reagent 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 nail polish or hot wax to prevent the coverslip from moving.

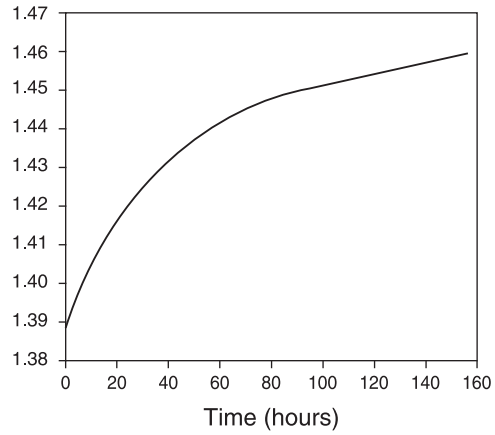

Figure 2. Increase in the refractive index of ProLong® Gold antifade reagent during the curing process.

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 may take longer to dissolve depending on how well it has cured. If using *SlowFade*® Gold, 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® Gold and *SlowFade*® Gold antifade reagents do improve slightly the longer they are in contact with the specimen. ProLong® Gold reagent in particular achieves maximum effectiveness once it has cured. When properly stored, samples mounted in ProLong® Gold or *SlowFade*® Gold antifade reagent 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® Gold and *SlowFade*® Gold reagents.*

Dye	ProLong® Gold Reagent	*SlowFade*® Gold Reagent
Alexa Fluor® 350	65%	55%
Alexa Fluor® 488	85%	90%
Alexa Fluor® 546	67%	76%
Alexa Fluor® 555	80%	89%
Alexa Fluor® 568	93%	88%
Alexa Fluor® 594	92%	92%
Alexa Fluor® 633	99%	ND
Alexa Fluor® 635	100%	85%
Alexa Fluor® 647	100%	100%
Alexa Fluor® 660	94%	99%
Alexa Fluor® 680	96%	99%
7-Aminoactinomycin D	92%	91%
Cy2	89%	83%
Cy3	83%	83%
Cy5	100%	100%
Fluorescein	85%	78%
BODIPY® FL	54%	80%
Tetramethylrhodamine	98%	75%
Texas Red®	61%	75%
DAPI	67%	69%
SYTOX® Green	85%	77%
TO-PRO®-3	92%	72%

*Expressed as percentage of initial fluorescence intensity remaining following 30 seconds of continuous illumination using a 40X/1.3 NA objective and a 100-watt Hg-arc lamp as the light source. ND = Not determined.

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

Cat. no.	Product Name	Unit Size
P36930	ProLong® Gold antifade reagent	10 mL
P36934	ProLong® Gold antifade reagent *special packaging*	5 × 2 mL
P36931	ProLong® Gold antifade reagent with DAPI	10 mL
P36935	ProLong® Gold antifade reagent with DAPI *special packaging*	5 × 2 mL
S36936	*SlowFade*® Gold antifade reagent	10 mL
S36937	*SlowFade*® Gold antifade reagent *special packaging*	5 × 2 mL
S36938	*SlowFade*® Gold antifade reagent with DAPI	10 mL
S36939	*SlowFade*® Gold antifade reagent with DAPI *special packaging*	5 × 2 mL

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