ProLong™ Gold and Diamond Antifade Mountants

Doc. Part No. MP36930 Pub. No. MAN0002469 Rev. F.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Contents and storage

Material	Product No.	Amount	Storage[1]
ProLong™ Gold Antifade Mountant	P36934	5 × 2 mL	Room temperature (15-30°) Protect from light.
	P10144	2 mL	
	P36930	10 mL	
ProLong™ Gold Antifade Mountant with DAPI [2]	P36935	5 × 2 mL	
	P36941	2 mL	
	P36931	10 mL	
ProLong™ Gold Antifade Mountant with SYTOX™ Deep Red [3]	P36987-5X2ML	5 × 2 mL	
	P36987	2 mL	
	P36988	10 mL	
ProLong™ Diamond Antifade Mountant	P36961	5 × 2 mL	2–8°C Protect from light.
	P36965	2 mL	
	P36970	10 mL	
ProLong™ Diamond Antifade Mountant with DAPI [2]	P36962	5 × 2 mL	
	P36966	2 mL	
	P36971	10 mL	
ProLong™ Diamond Antifade Mountant with SYTOX™ Deep Red [3]	P36990-5X2ML	5 × 2 mL	–20°C Protect from light.
	P36990	2 mL	
	P36991	10 mL	

[1] The product may also be stored at ≤–20°C. When stored as directed, the product is stable for at least 6 months.
[2] DAPI stains the cellular nucleus and has an Ex/Em of 360/460nm that can be imaged with a traditional DAPI filter set.
[3] SYTOX™ Deep Red stains the cellular nucleus and has an Ex/Em of 660/682 nm that can be imaged using a traditional Cy5™/Deep Red filter set.

Product description

ProLong™ Gold and Diamond Antifade Mountants are hard (curing) mountants that achieve an optimal refractive index of 1.46 after curing and can be used for long-term storage of slides.

ProLong™ Diamond Mountant causes little or no quenching of fluorescent signal after mounting and 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 Table 1). ProLong™ Gold Mountant can be conveniently stored at room temperature and provides a similar antifade protection for Alexa Fluor™ dyes. When you need to archive the slides for later use, we recommend using the ProLong™ Diamond or Gold Antifade Mountants, which can save the fluorescent signal for weeks or even months, depending upon sample conditions.

For RIMS (Refractive index matching solution) mountants with a refractive index of 1.52, look into ProLong™ Glass Antifade Mountants (Cat no. P36980) for curing mountant, or SlowFade™ Glass Antifade Mountant (Cat no. S36916) for non-curing mountant.

All ProLong™ mountants are available with DAPI or SYTOX™ Deep Red in the mounting medium to eliminate the need for a separate nuclear counterstaining step.

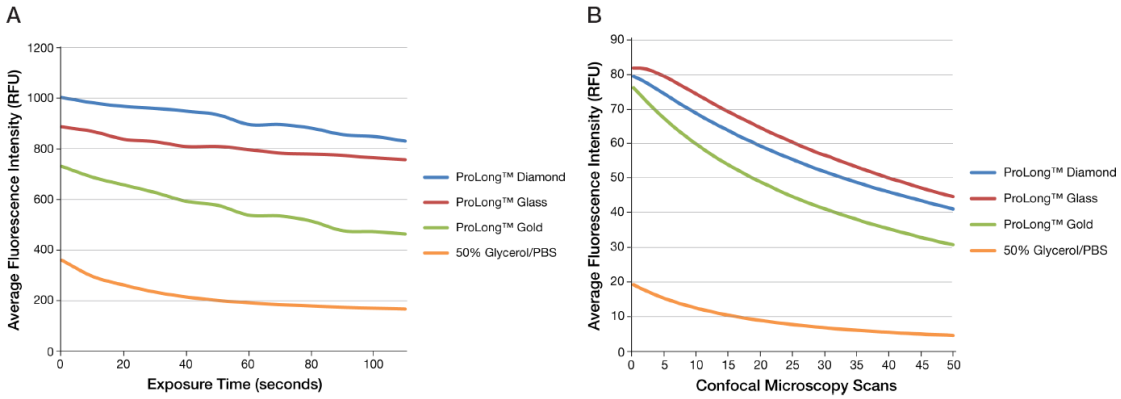

Fig. 1 FITC photobleach curves with widefield (A) and confocal (B) microscopes.

(A) Tubulin in HeLa cells was labeled with mouse anti-tubulin primary antibody, detected with fluorescein (FITC)-labeled goat anti-mouse antibody, and mounted with PBS + 50% glycerol or various commercially available antifade mounting media. Photobleach curves were collected by illuminating the samples for 2 minutes using a 100-watt Hg-arc lamp, imaged using a 20x air objective, then acquired using a 12-bit monochrome camera. The data plotted is the mean florescence intensity of three fields of view over time.

(B) Tubulin in HeLa cells were labeled with mouse anti-tubulin primary antibody, detected with fluorescein (FITC)-labeled goat anti-mouse antibody, and mounted with PBS + 50% glycerol or various commercially available antifade mounting media. Photobleach curves were collected using a confocal microscope with a 20x air objective scanning regions of interest fifty times with a pixel dwell time of 1.6 µs. Excitation source power intensity was set such that ProLong™ Diamond retained 50% of initial signal intensity at the end of the final scan. Detector gain was held constant for all mounting media. Plotted data is the mean fluorescence intensity from fifteen regions of interest across mounted samples as number of scans.

Before you begin

Important information for ProLong™ antifade mountants

Be sure to closely follow the instructions in this guide when using the ProLong™ Gold or Diamond Antifade Mountant.

- Ensure the mountant is at room temperature by letting stand on the benchtop for at least 1 hour.
- If you notice solidifying of the mountant in the vial, warm the vial to a minimum temperature of 37°C (optimally 45-50°C) for 15-30 minutes in a water bath to dissolve the gel. The mountant should function normally after the gel is dissolved. Close the lid properly in between uses to prevent evaporation and solidification of mountant in the vial and store as recommended.
- Before adding mountant, remove excess moisture from the slide by tapping the side of the slide or coverslip on a clean laboratory wipe.
- Cure the sample after 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, 4°C, or optimally 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 Diamond Antifade Mountants

- ProLong™ Gold = pH 7.4 at 20°C
- ProLong™ Diamond = pH 8.4 at 20°C
- Refractive index gradually increases as it cures (Figure 2).
- ProLong™ mountants are useful for long-term storage (many months if edges are sealed), but they must be cured for optimum performance.
- For a non-curing mountant with a 1.52 refractive index, try SlowFade™ Glass Antifade Mountant (Cat. no. S36916), which enables sharp imaging in specimens with thickness of up to 500 µm.
- For tissues 500 µm to a few centimeters thick, or hard to clear tissues (e.g., heart, liver, etc.), CytoVista™ Tissue Clearing/Staining Kit (Cat. no. V11324) is highly recommended.
- For microplate imaging, including high-content imaging, CytoVista™ 3D Cell Culture Clearing/Staining Kit (Cat. no. V11325) is recommended.

- For plant tissue clearing and mounting, Image-iT™ Plant Tissue Clearing Reagent (Cat. no. V11328) is recommended.

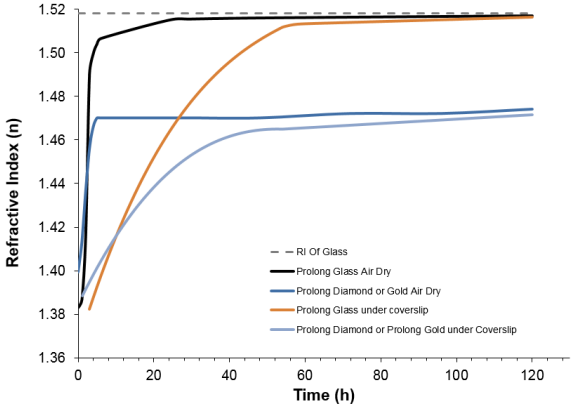

Fig. 2 ProLong™ Antifade Mountant curing time for final refractive index. The refractive indices of various ProLong™ Antifade Mounting Media were measured using a Thermo Scientific™ ABBE-3L refractometer. For this analysis, 300 µL of mountant was applied to cover the measuring prism surface. Mountants were cured open to air at room temperature or under a coverslip for various time periods. These plotted data are a close approximation of the change in refractive index either in open air or under a coverslip. Depending on the air humidity level or the temperature during curing or thickness of sample, RI and curing time can vary.

Experimental protocol

Mount samples

Note: 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. If needed, allow the vial to equilibrate to room temperature.
2. 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.
3. For samples mounted using the ProLong™ Gold or 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.

   After sealing, store the slide in a covered slide box at room temperature, 4°C, or optimally ≤–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.

Remove mounted coverslips

Note: If your workflow requires the removal of coverslips for further manipulation or staining of the specimen, we highly recommend that you use non-curing mounting media such as SlowFade™ Diamond, SlowFade™ Gold or SlowFade™ Glass Antifade Mountants.

1. 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.
2. *(Optional)* If any sealing material has been used, remove it prior to processing.
3. After removal, wash the sample well with PBS before continuing to remove any residual mounting medium.

Fluorescence microscopy

Note: If ProLong™ Diamond Antifade Mountant with DAPI or ProLong™ Gold Antifade Mountant with DAPI is used, then the cellular nucleus can be imaged using a fluorescence microscope with a traditional DAPI filter set. DAPI is a dsDNA stain with an Ex/Em of 360/460nm.

Note: If ProLong™ Diamond Antifade Mountant with SYTOX™ Deep Red or ProLong™ Gold Antifade Mountant with SYTOX™ Deep Red is used, then the cellular nucleus can be imaged using a fluorescence microscope with a traditional Cy5™/Deep Red filter set. SYTOX™ Deep Red is a dsDNA stain with an Ex/Em of 660/682 nm that shows minimum fluorescence in the absence of dsDNA or presence of RNA or ssDNA, thus having a very low ex-nucleus background.

Samples may be examined with a fluorescence microscope before the mounting medium dries. However, the antifade properties of ProLong™ Diamond Antifade Mountants do improve with curing. ProLong™ Diamond Antifade Mountant in particular achieves maximum effectiveness once it has cured. When properly stored, samples mounted in ProLong™ Diamond or ProLong™ Gold 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.

Troubleshooting

Observation	Possible cause	Recommended action
Low specific signal or high background in ICC or IHC experiments	Enhance the specific signal over background.	Use fluorescence-grade fixation and permeabilization reagents, such as provided in the Image-iT™ Fixation/Permeabilization Kit (Product No. R37602).
		Reduce the nonspecific background using a good blocker. Some of the blockers are mentioned as ReadyProbes™ imaging accessories at thermofisher.com/readyprobes-ready-to-use-imaging-reagents.
		Titrate and optimize the concentration of primary and secondary antibodies. ICC- and IHC-compatible primary and secondary antibodies can be searched at using the antibody search tool at thermofisher.com/antibodies.
		If autofluorescence is noticed, specifically for tissue, then use the following techniques. Depending on the cause of autofluorescence, these techniques may or may not work for particular tissues or tissue preparations. • After fixation and permeabilization of tissue, incubate tissue for 7 minutes at room temperature with freshly made solution of either 0.1 M glycine or 0.1% (w/v) sodium borohydride. • If autofluorescence is observed due to aldehyde fixation, red-blood cells, or structural elements such as collagen and elastin, then use the ReadyProbes™ Tissue Autofluorescence Quenching Kit (Product No. R37630).
		If all of the above steps do not reduce the background enough to observe specific signal, then the signal can be enhanced using SuperBoost™ tyramide signal amplification available at thermofisher.com/tyramide-signal-amplification-tsa. SuperBoost™ tyramide signal amplification is known to enhance the signal up to 200-fold better then traditional ICC/IHC techniques.

Appendix

Table 1 Photobleach resistance of various fluorophores when mounted using ProLong™ Glass, Diamond, or Gold Antifade Mountants.

Fluorescent dye	Ex/Em (nm)	Resistance to photobleaching[1]		
		ProLong™ Glass	ProLong™ Diamond	ProLong™ Gold
Hoechst™	350/461	+++	+++	+++
DAPI	345/455	+++	+++	++
BODIPY™ FL	505/513	Not tested	+++	+
Alexa Fluor™ 488	495/519	+++	++	++
Alexa Fluor™ PLUS™ 488	495/519	+++	++	+
GFP	488/510	++	++	Not recommended
Fluorescein	494/518	+++	+++	+
Cy3™	550/570	++	++	++
Alexa Fluor™ 546	556/575	++	++	+++
Tetramethylrhodamine	555/580	++	+++	+
Alexa Fluor™ 555	555/565	+++	+++	+++
Alexa Fluor™ PLUS™ 555	555/565	+++	+++	++
TagRFP	555/584	++	++	Not recommended
mCherry	575/610	+++	+++	+++
Alexa Fluor™ 568	578/603	+++	+++	+
Texas Red™	595/615	+++	+++	+++
Alexa Fluor™ 594	590/617	+++	+++	+++
TO-PRO™-3	642/661	+++	+++	+
Alexa Fluor™ 647	652/668	+++	+++	+++
Alexa Fluor™ PLUS™ 647	652/668	+++	+++	+++
Cy5™	650/670	+++	+++	+++

[1] Photobleaching resistance was quantified on a Zeiss™ LSM 710 confocal microscope. HeLa or U2OS cells were stained and mounted using standard immunocytocehmistry (ICC) protocols. Five regions within three fields of view were scanned 15 times with 1.58 µs dwell time per pixel. Excitation wavelength and intensity were optimized by fluorophore. On an epi-fluoresence microsocope using 100-watt Hg-arc lamp, this amount of light/photon exposure will be equal to 60–90 seconds. In the table, "+++" designates when 80% or more of signal intensity was left, as compared to initial signal intensity. "++" designates 65–80% remaining signal intensity and "+" represents 50–65% remaining signal intensity. "Not recommended" means less than 50% remaining signal intensity.

Ordering information

Cat. No.	Product name	Unit size
P36934	ProLong™ Gold Antifade Mountant	5 × 2 mL
P10144	ProLong™ Gold Antifade Mountant	2 mL
P36930	ProLong™ Gold Antifade Mountant	10 mL
P36935	ProLong™ Gold Antifade Mountant with DAPI	5 × 2 mL
P36941	ProLong™ Gold Antifade Mountant with DAPI	2 mL
P36931	ProLong™ Gold Antifade Mountant with DAPI	10 mL
P36961	ProLong™ Diamond Antifade Mountant	5 × 2 mL
P36965	ProLong™ Diamond Antifade Mountant	2 mL
P36970	ProLong™ Diamond Antifade Mountant	10 mL
P36962	ProLong™ Diamond Antifade Mountant with DAPI	5 × 2 mL
P36966	ProLong™ Diamond Antifade Mountant with DAPI	2 mL
P36971	ProLong™ Diamond Antifade Mountant with DAPI	10 mL
Related Products		
S36937	SlowFade™ Gold Antifade Mountant	5 × 2 mL
S36936	SlowFade™ Gold Antifade Mountant	10 mL
S36939	SlowFade™ Gold Antifade Mountant with DAPI	5 × 2 mL
S36938	SlowFade™ Gold Antifade Mountant with DAPI	10 mL
S36963	SlowFade™ Diamond Antifade Mountant	5 × 2 mL
S36967	SlowFade™ Diamond Antifade Mountant	2 mL
S36972	SlowFade™ Diamond Antifade Mountant	10 mL
S36964	SlowFade™ Diamond Antifade Mountant with DAPI	5 × 2 mL
S36968	SlowFade™ Diamond Antifade Mountant with DAPI	2 mL
S36973	SlowFade™ Diamond Antifade Mountant with DAPI	10 mL
S36917-5X2ML	SlowFade™ Glass Antifade Mountant	5 × 2 mL
S36917	SlowFade™ Glass Antifade Mountant	2 mL
S36918	SlowFade™ Glass Antifade Mountant	10 mL
S36920-5X2ML	SlowFade™ Glass Antifade Mountant with DAPI	5 × 2 mL
S36920	SlowFade™ Glass Antifade Mountant with DAPI	2 mL
S36921	SlowFade™ Glass Antifade Mountant with DAPI	10 mL
S11380	SYTOX™ Deep Red Nucleic Acid Stain	50 µL
S11381	SYTOX™ Deep Red Nucleic Acid Stain	5 × 50 µL

Limited product warranty

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