ProLong® Antifade Kit (P7481)

Quick Facts

Storage upon receipt:

- ≤–20ºC
- Protect from light
- Protect from air

Notes: The ProLong® antifade reagent should be mixed with the ProLong® mouting medium *just* prior to mounting the samples.

Introduction

When exposed to excitation light, all fluorescent dyes fade (photobleach). The degree of photobleaching depends on both the intensity and the duration of illumination. The photon output of a dye represents the average number of cycles of excitation followed by fluorescence emission that the dye undergoes before it is irreversibly photobleached. The average photon output is defined by the ratio of the probability that the dye will fluoresce (fluorescence quantum efficiency or Q_f) and the probability that it will photoreact irreversibly to become a nonfluorescent species (photobleaching quantum efficiency or Q_b). For example, fluorescein, which is very photolabile, has a Q_f/Q_b of about 30,000 in alkaline solution.[1] Both Q_f and Q_b are properties of the dye that may be affected significantly by the dye's environment. The primary environmental influence on Q_b is the presence of singlet oxygen and free radical species. The main purpose of any antifade reagent is to sustain dye fluorescence. This is usually accomplished by inhibiting the generation and diffusion of reactive oxygen species, thereby reducing Q_b (preferably without any accompanying decrease in Q_f), so that fluorescence will persist.

Molecular Probes' ProLong® Antifade Kit (P7481) outperforms all other commercially available antifade reagents and causes little or no quenching of the fluorescent signal. For example, ProLong® antifade reagent provides excellent fluorescence stabilization when used to mount fluorescein-stained HEP-2 cells and clearly outperforms a popular *p*-phenylenediamine–containing antifade reagent [2] (Figure 1). ProLong® reagent's performance on Texas Red® conjugate–stained cells is even more dramatic. ProLong® antifade reagent not only protects Texas Red® conjugates from photobleaching, but also unquenches the fluorescence, and the signal becomes noticeably brighter (Figure 1B). ProLong® antifade reagent also inhibits the fading of tetramethylrhodamine, as well as the fading of DNA-bound nucleic dyes

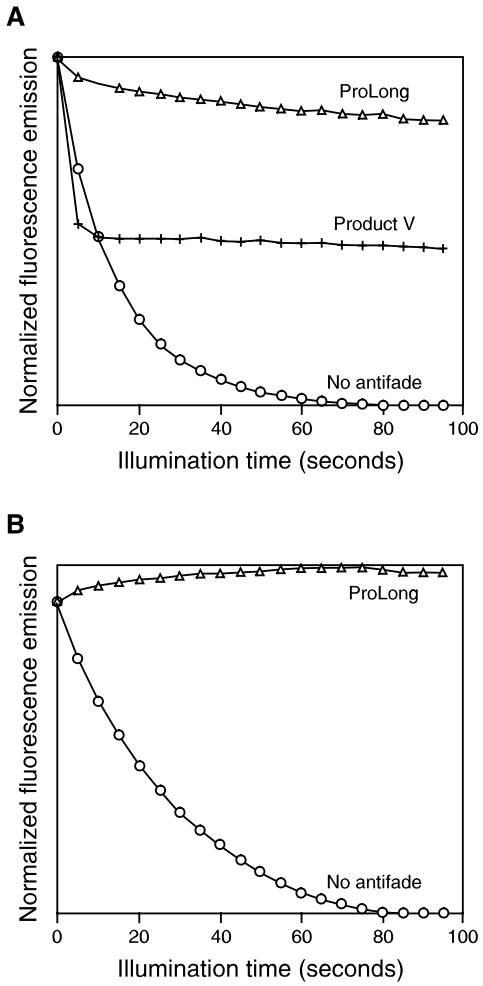

Figure 1. Bleaching profiles of cells labeled with fluorescein (Panel A) or Texas Red® conjugates (Panel B). In these photobleaching experiments, human epithelium cells (Hep2) were probed with human anti-nuclear antibodies and then developed for visualization with fluorophore-labeled secondary reagents. The data were collected through a 40X objective with a Star-1 cooled CCD camera (Photo-metrics Ltd.) and analyzed with an Image-1/AT image processor (Universal Imaging). Identical samples were mounted in ProLong® antifade reagent (△), Product V (+) or medium containing no antifade reagent (○). Although these data were normalized, we observed little or no quenching of samples mounted with the ProLong® mounting medium.

such as DAPI, propidium iodide, and YOYO®-1—again without significantly quenching their fluorescence.

For maximum resistance to photobleaching, we now offer ProLong® Gold antifade reagent (P36930, P36934). The ProLong® Gold formulation comes 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. Like the original ProLong® antifade reagent, ProLong® Gold reagent will cure

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, making it an especially valuable tool for multicolor applications. For convenience, we also offer ProLong® Gold antifade reagent with DAPI (P36931, P36935); the addition of DAPI in the mounting medium eliminates the need for a separate nuclear counterstaining step. A detailed protocol for ProLong® Gold reagent is available on our website at probes.invitrogen.com.

Note: ProLong® reagent's compatibility with a multitude of dyes and dye complexes makes it an especially valuable tool for multicolor *in situ* hybridization. However, in some preparations, ProLong® antifade reagent may cause unstained tissue and cells to appear blue when viewed through a 7-amino-4-methylcoumarin (AMCA) or Cascade Blue® filter set. Also, the ProLong® antifade reagent may not protect BODIPY® fluorophores against photobleaching.

Materials

ProLong® Antifade Kit Contents

- **Powdered ProLong® antifade reagent** (Component A), 20 vials of powdered reagent
- **ProLong® mounting medium** (Component B), two dropper bottles containing 15 mL each. One mL of this medium should be mixed with the contents of each vial of ProLong® antifade reagent just prior to mounting the samples.

Storage and Handling

When stored at room temperature protected from light, ProLong® antifade reagent (Component A) is stable for at least six months and ProLong® mounting medium (Component B) is stable for at least three months. To increase the shelf life of the ProLong® mounting medium, store it at ≤–20°C, preferably in single-use aliquots under argon or nitrogen gas. The vials of ProLong® antifade reagent can also be stored at ≤–20°C without compromising performance; however, freezer storage is not required for stability. The ProLong® antifade reagent is moisture sensitive; if stored at ≤–20°C, warm the vials to room temperature before opening.

Once the bottle has been opened, ProLong® mounting medium that has been stored at room temperature may gel or turn milky with time. However, the optical clarity and fluorescence intensity of preparations mounted in this milky medium are no different than that of preparations mounted in clear medium. The milky medium may be cleared by heating the bottle containing the medium in a 50°C water bath for approximately one hour or until cloudiness disappears. Alternatively, gelled or milky medium may be heated in a microwave on low power for 10 seconds. Do not microwave the medium at higher power or for longer than 10 seconds because the bottle may melt. Allow the medium to cool to room temperature before using.

Note: ProLong® antifade reagent should be mixed with the ProLong® mounting medium just prior to mounting the samples. *Do not mix the two components in this kit until the sample is ready for mounting.*

Application

General Considerations

The ProLong® Antifade Kit is for nonliving specimens only, including fixed cells, tissues, and cell-free preparations. The effects of ProLong® antifade reagent on binding affinities of dyes and ligands is currently undetermined.

Note: Once the two components in the ProLong® Antifade Kit are mixed, the ProLong® antifade reagent will begin to slowly degrade, producing a brown precipitate. However, once the mixture of mounting medium and antifade reagent is dry, the ProLong® antifade reagent no longer degrades. When properly stored, slides that have been mounted with freshly prepared ProLong® reagent remain free of discoloration. Although the mixture of ProLong® antifade reagent and mounting medium is still effective several hours after it is prepared, the brown precipitate may diminish the fluorescence of the sample, particularly of the longer-wavelength fluorophores. The mixture of antifade reagent and mounting medium should be used as soon as possible after mixing; any portion of the mixture that remains at the end of the day should be discarded.

Protocol for Mounting Samples

1. Once the sample is ready for mounting, add approximately 1 mL of ProLong® mounting medium (Component B) to one of the brown vials containing the ProLong® antifade reagent (Component A). The ~1 mL volume of ProLong® mounting medium can be estimated either by applying ~32 drops from the dropper bottle or by using a 1 mL–pipetting device. For using a pipettor, the tip of the dropper bottle must first be removed, and, since the mounting medium is viscous, it may help to snip off the end of the disposable pipet tip to increase its diameter. Mix the two components by gently pipetting the mounting medium up and down until the antifade reagent no longer adheres to the sides of the brown vial. Continue mixing either by pipetting or by vortexing. Remove any bubbles that have formed during the mixing procedure by sonication.

2. Apply the antifade reagent/mounting medium mixture to a specimen that is almost dry. Prior to mounting the sample, remove residual liquid from the sample. Then, using a Pasteur pipet, apply the mixture and place a coverslip over the specimen. Because the ProLong® antifade reagent will no longer degrade once the mounting medium has dried, apply only a small volume of the mixture, thereby ensuring that the sample dries quickly. DO NOT SEAL THE COVERSLIP TO THE SLIDE UNTIL THE MOUNTING MEDIUM HAS DRIED.

3. To dry the slide, place it on a flat surface in the dark. Drying 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. Once dry, seal the coverslip to the slide in order to prevent shrinkage of the mounting medium and subsequent sample distortion. After sealing, store the slide upright in a covered slidebox at ≤–20°C. Desiccant may be added to the box to ensure that the slide remains dry.

Fluorescence Microscopy

Samples may be examined with a fluorescence microscope before the mounting medium dries. However, the ProLong® antifade reagent may be more potent once the sample is allowed to dry, and drying may enhance the resolution of the staining as well. When properly stored, samples mounted in ProLong® antifade reagent continue to resist photobleaching long after they are mounted.

To further reduce photobleaching, minimize the exposure of fluorescently labeled specimens to light with neutral density filters and expose samples only when observing or recording a signal. Maximize collection of fluorescence 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.

References

1. *Applications of Fluorescence in the Biomedical Sciences*, D.L. Taylor *et al*., Eds., Alan R. Liss (1986) pp. 129–140; **2.** J Histochem Cytochem 41, 1833 (1993).

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

Cat #	Product Name	Unit Size
P7481	ProLong® Antifade Kit	1 kit
P36930	ProLong® Gold antifade reagent	10 mL
P36934	ProLong® Gold antifade reagent *special packaging*	5 X 2 mL
P36931	ProLong® Gold antifade reagent with DAPI	10 mL
P36935	ProLong® Gold antifade reagent with DAPI *special packaging*	5 X 2 mL

Contact Information

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Please visit our Web site — **probes.invitrogen.com** — for the most up-to-date information.

Molecular Probes, Inc.
29851 Willow Creek Road, Eugene, OR 97402
Phone: (541) 465-8300 • Fax: (541) 335-0504

Customer Service: 6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338 • Fax: (541) 335-0305 • probesorder@invitrogen.com

Toll-Free Ordering for USA:
Order Phone: (800) 438-2209 • Order Fax: (800) 438-0228

Technical Service: 8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353 • Toll-Free (800) 438-2209
Fax: (541) 335-0238 • probestech@invitrogen.com

Invitrogen European Headquarters
Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100 • Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com