

# ProLong™ Live Antifade Reagent

Catalog nos. P36974, P36975

Table 1. Contents and storage

Product*	Amount	Catalog no.	Storage†
ProLong™ Live Antifade Reagent	5 × 1 mL	P36974	<ul style="list-style-type: none"> <li>• ≤ -20°C or 2–8°C</li> <li>• Protect from light</li> </ul>
	1 mL	P36975	
* Product is not sterile but produced using aseptic conditions, which includes filtering through 0.1-µm filter.			
† When stored at ≤ -20°C, the product is stable for at least 6 months with up to 4 freeze-thaw cycles. When stored at 2–8°C, the product should be used within 30 days (a precipitate may form at this temperature).			

## Introduction

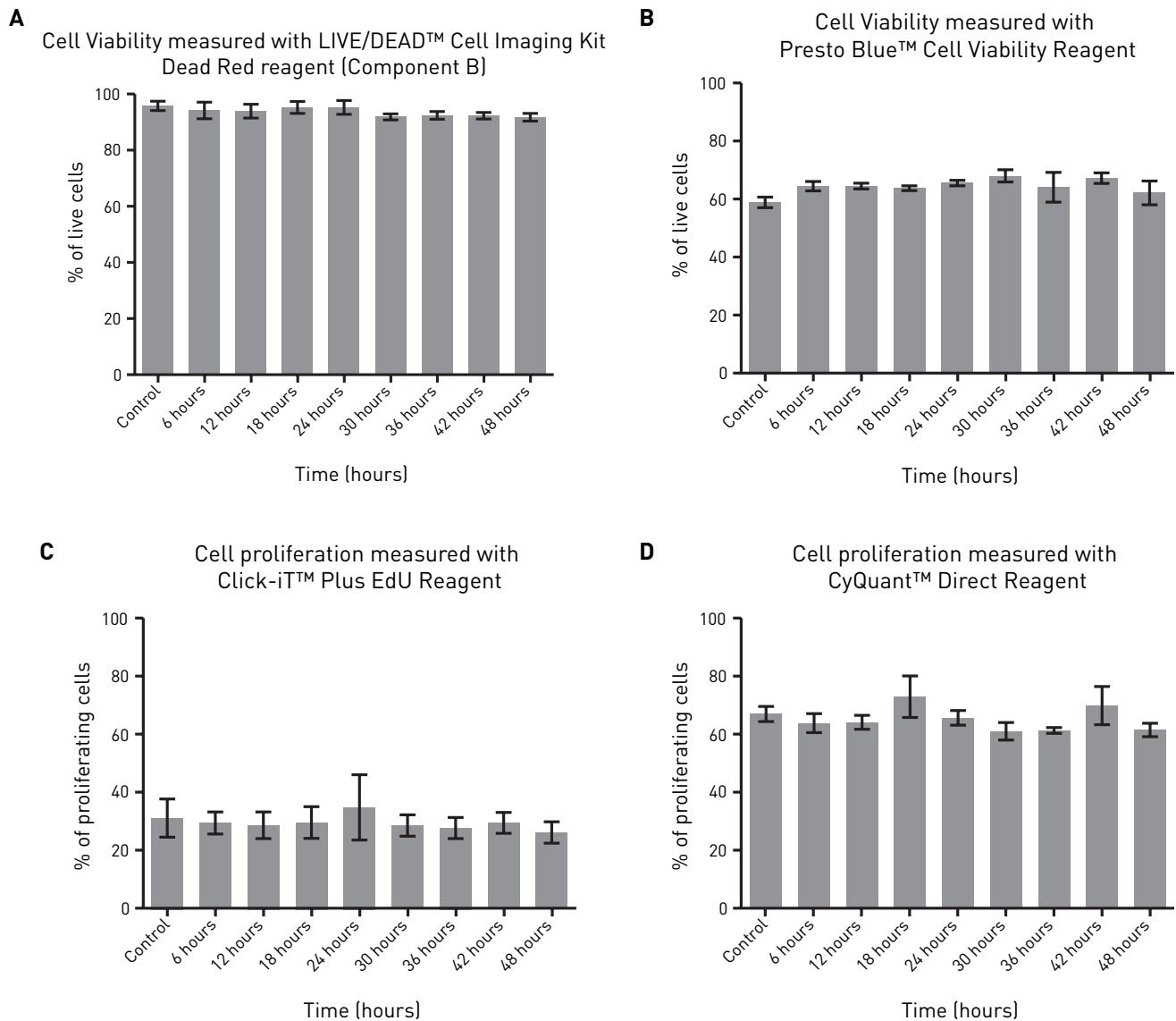
Loss of fluorescence signal due to photobleaching can lead to a significant loss of sensitivity for an assay. This loss in sensitivity is even more pronounced when trying to detect low-abundant or rare targets within a cell or tissue. ProLong™ Live Antifade Reagent offers protection against the effects of photobleaching in live cells.

ProLong™ Live Antifade Reagent utilizes Oxyrase™ technology<sup>1</sup> and contains enzymes from the plasma membrane of naturally occurring *E. coli*. These enzymes are known for their ability to reduce photobleaching and photodamage in live-cell fluorescent imaging applications.<sup>2–4</sup> ProLong™ Live Antifade reagent can metabolize free radical singlet oxygen to reduce photobleaching without affecting intracellular functions. As shown in Figure 1 (page 2), the presence of ProLong™ Live has minimum effects on cellular viability or proliferation. ProLong™ Live reagent provides antifade protection against most fluorescent reagents frequently used for live-cell fluorescent imaging, such as GFP, RFP, Hoechst™ 33342, as well as MitoTracker™, LysoTracker™, and CellTracker™ reagents (Table 2 and Figure 2, page 3). ProLong™ Live reagent can be added to any cell culture medium or buffer suitable for fluorescent imaging.

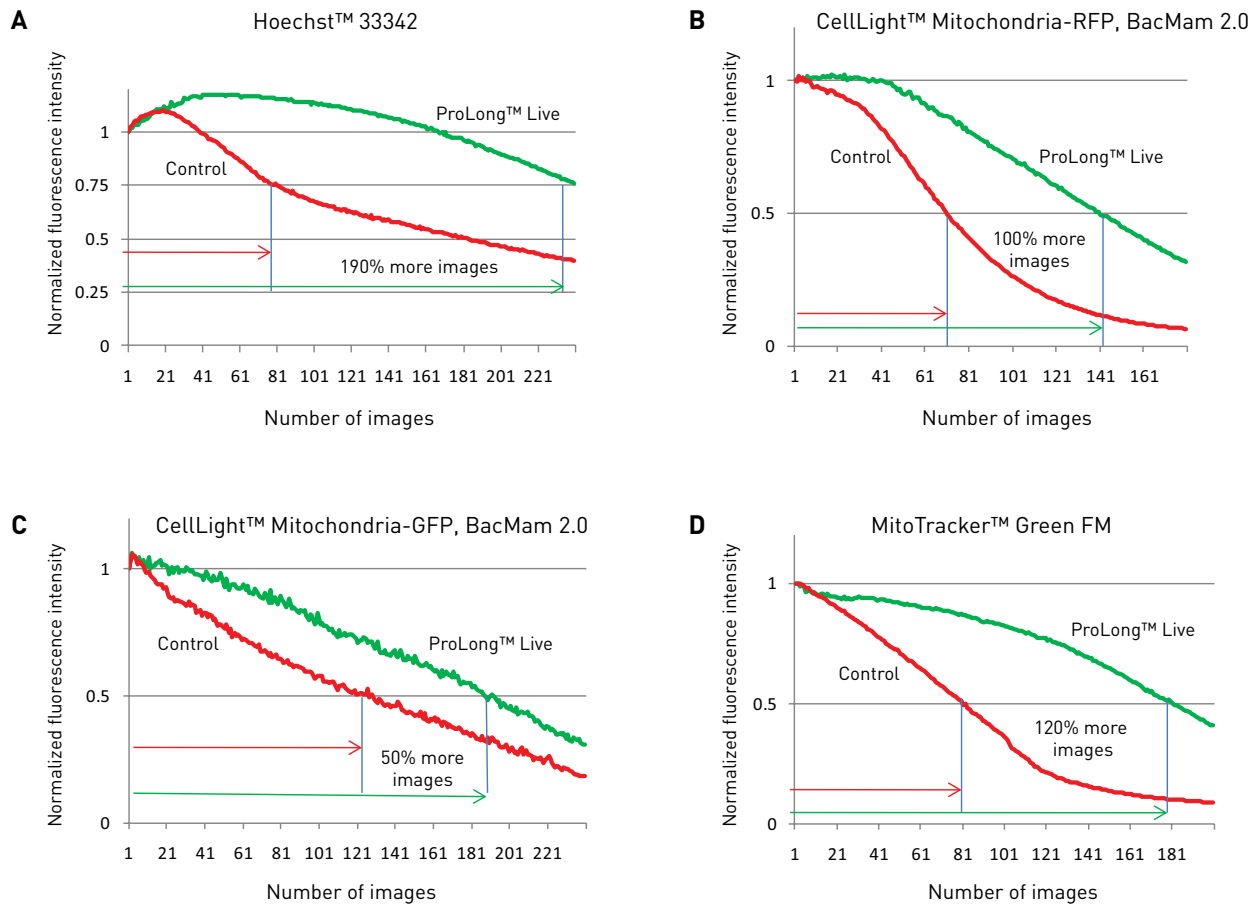
In addition to the use of the ProLong™ Live Antifade Reagent, further steps can be taken to reduce photobleaching by illuminating the sample only when needed, using neutral density filters to reduce light exposure when setting up for a scan, and choosing the most photostable dyes for the application. If protection against photobleaching is needed in fixed cells, we recommend using ProLong™ Diamond Antifade Mountant, when curing is required, and SlowFade™ Diamond Mountant, when a non-curing mountant is required.

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**Figure 1. Cell proliferation and viability in the presence of ProLong™ Live Antifade Reagent.** HeLa cells were plated at concentration of 1000 cells/well in a 96-well plate, using MEM media (Cat. no. 11095-080) with 10% FBS (Cat. no. 16000-036). A working concentration of ProLong™ Live Antifade Reagent (Cat. no. P36974) was added to subsequent 8 replicate wells, every 6 hours, for up to 48 hours. Cell viability was detected using **(A)** Live/Dead™ Dead Red (Cat. no. R37601) and **(B)** Presto Blue™ reagents (Cat. no. A13261), and cell proliferation was detected with **(C)** Click-iT™ Plus EdU (Cat. no. C10639) and **(D)** CyQuant™ Direct reagents (Cat. no. C7026) using Thermo Scientific™ ArrayScan™ VTI HCS Reader (Cat. no. NX100002L). These data demonstrate that the presence of ProLong™ Live Antifade Reagent has no statistically significant effect on cell proliferation and viability. Error bars = standard deviation.



**Figure 2. Photobleach protection for other key dyes and fluorescent proteins.** HeLa or U2OS cells were stained with **(A)** Hoechst™ 33342 [Cat. no. H21492], **(B)** CellLight™ Mitochondria-RFP [Cat. no. C10505], **(C)** CellLight™ Mitochondria-GFP [Cat. no. C10508], or **(D)** MitoTracker™ Green FM [Cat. no. M7514] reagents. Sample were either left in complete medium (control) or ProLong™ Live Antifade Reagent [Cat. no. P36974] was added to the complete medium. Cells were incubated for 2 hours in the dark. At the end of incubation, cells were imaged for 15 seconds with optimal but consistent Em/Ex imaging conditions using Thermo Scientific™ ArrayScan™ VTI HCS Reader [Cat. no. NX100002L]. Normalized fluorescence intensity was determined using Cellomics software.



**Table 2.** Photobleach protection for other key dyes and fluorescent proteins.

	% of signal intensity remaining after 100 images		Number of images before photobleaching reduced the signal to half	
	Control (media only)	ProLong™ Live in media	Control (media only)	ProLong™ Live in media
Hoechst™ 33342	60%	100%	80†	230†
emGFP	60%	80%	120	190
MitoTracker™ Green	35%	80%	80	180
TagRFP	25%	70%	70	140
MitoTracker™ Red	35%	55%	70	120
MitoTracker™ Deep Red	25%	60%	60	120

† For the Hoechst™ 33342 dye, the number of images demonstrated is when the normalized fluorescence intensity was reduced to 75% of the initial value.

## Before you begin

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### Storage and handling

ProLong™ Live reagent can be stored at 2–8°C for up to 30 days. For longer storage, store the reagent at ≤–20°C for up to 6 months.

If stored frozen, thaw the ProLong™ Live reagent at room temperature or in an ice water bath until the ice melts. While in use, the product should be kept in an ice water bath.

**Do not exceed 37°C while thawing or using the reagent.**

### Prepare working imaging solution

**IMPORTANT!** Mix stock and working solutions gently. Do not vortex or agitate vigorously.

**Note:** During regular freeze/thaw cycles, a small amount of precipitation will form in the reagent tube. To remove this precipitate, centrifuge the tube at ~500–700 × g or at 2000–3000 rpm for 5 minutes before each use. To avoid repeated freeze/thaw cycles, ProLong™ Live reagent can be divided into single-use aliquots for –20°C or it can be stored at 2–8°C for 30 days.

To prepare ProLong™ Live working solution, dilute the reagent 1:50 to 1:100 in any imaging solution, including complete culture medium or an isotonic buffer such as PBS. For best fluorescent imaging results, we recommend Live Cell Imaging Solution (Cat. no. A14291DJ) or FluoroBrite™ DMEM Media (Cat. no. A1896701). In some situations, a small amount of precipitation may be observed, but this will not affect performance.

**Note:** To enhance the performance of ProLong™ Live reagent, you can add additional sodium DL-lactate to the imaging working solution at a final concentration of 10–20 mM.

**Note:** If additional sterilization is required, you may filter the working solution through a 0.2-µm low-protein binding filter such as the Acrodisc™ PF Syringe filter with a 0.8-µm prefilter and a 0.2-µm final filter (Pall Life Sciences, Cat. no. 4187). To compensate for any losses during filtration, we recommend using a 1:50 to 1:75 dilution while preparing the working solution. Antibiotics such as a mixture of Penicillin-Streptomycin can also be added to media without affecting the performance of ProLong™ Live reagent.

## Experiment guidelines

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1. Stain the cells with the desired fluorescent dye and/or use cells expressing a desired fluorescent protein.
2. Wash the cells with 1× PBS.
3. Add imaging working solution to the cells.
4. Incubate the cells in dark for 15 minutes to 2 hours.

**Note:** For best performance, 2 hour incubation is recommended.

5. Image or analyze the cells using the desired instrument settings.

**Note:** We do not recommend leaving the ProLong™ Live solution on live cells for more than 24 hours. If more than 24 hours is needed for analysis, we recommend keeping the cells in culture medium without ProLong™ Live reagent in between imaging procedures.

## References

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1. Lab Medicine 31(9), 509–512 (2000); 2. Cell motility and the cytoskeleton, 32(3), 173–186 (1995); 3. Journal of Cell Science, 110(21), 2635–2645 (1997); 4. Journal of cell science 118(18), 4113–4122 (2005).

## Product List

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

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Cat. no.	Product Name	Unit Size
P36974	ProLong™ Live, Antifade Reagent	5 × 1 mL
P36975	ProLong™ Live, Antifade Reagent	1 mL

### Related Products

A14291DJ	Live Cell Imaging Solution	500 mL
A1896701	FluoroBrite™ DMEM	500 mL
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

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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.

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