ProLong™ Glass Antifade Mountant

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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**.

Product description

ProLong™ Glass Antifade Mountant is a glycerol-based, hard-setting, and ready-to-use mountant that is applied directly to fluorescently-labeled cells or tissue samples on microscope slides. ProLong™ Glass Antifade Mountant is a refractive index matching solution (RIMS) with a refractive index (RI) of ~1.52 after curing (Figure 1), which matches closely to glass coverslips and oil-immersion microscope objectives. This allows for increased resolution and sensitivity by minimizing refraction of the light path, which maximizes the light gathering potential of the microscope (Figure 2). This better match of refractive index with cellular/tissue components also allows some optical clarity, allowing viewing of thicker sections of tissue, organoids, and spheroids.

ProLong™ Glass Antifade Mountant is designed to provide unparalleled antifade protection across the entire visible and near-infrared spectra (Figure 4; Table 2). It can be used with most fluorescent dyes or fluorescent proteins (e.g., GFP, RFP, mCherry) and any cell/tissue types from a depth of 0 µm to 150 µm for bright, high-resolution Z-stack, 3-D, and 2-D images (Figure 3). Furthermore, this mountant does not discolor or extensively shrink for most sample types when cured, making it possible to take high-quality fluorescent images weeks, or even months, after mounting the slides.

Benefits of ProLong™ Glass Antifade Mountant include:

- A high refractive index of ~1.52 after curing (equal to glass coverslips) and compatibility with immersion oil and oil-immersion microscope objectives.
- Improved resolution when imaging with oil immersion objectives on confocal microscope objectives with confocal laser scanning microscopy (CLSM) and other high-resolution microscopes.
- Protection of fluorescent dyes and fluorescent proteins from photobleaching across the entire visible and near-infrared spectra.

Note: 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.

Contents and storage

Material	Catalog No.	Amount	Storage[1,2]
ProLong™ Glass Antifade Mountant	P36980	5 × 2 mL	• Store at 2–8°C upon receipt. • Avoid freeze/thaw cycles. • Protect from light.
	P36982	2 mL	
	P36984	10 mL	
ProLong™ Glass Antifade Mountant with NucBlue™ (Hoechst 33342) [3]	P36981	5 × 2 mL	
	P36983	2 mL	
	P36985	10 mL	
ProLong™ Glass Antifade Mountant with SYTOX™ Deep Red Nucleic Acid Stain[4]	P36993-5X2ML	5 × 2 mL	• Store at –20°C upon receipt. • Avoid freeze/thaw cycles. • Protect from light.
	P36993	2 mL	
	P36994	10 mL	

[1] Product can be stored at –20°C for longer term storage. When stored as directed, the product is stable for at least 6 months from the date of receipt.

[2] Product will freeze or become more viscous at temperatures below 0°C.

[3] NucBlue™ (Hoechst 33342) stains the cellular nucleus with an Ex/Em of 350/461nm that can be imaged with a traditional DAPI filter set.

[4] SYTOX™ Deep Red stains the cellular nucleus with an Ex/Em of 660/682 nm that can be imaged using a traditional Cy5™/Deep Red filter set.

Materials required but not provided

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Source [1]
Cells/Tissue	MLS Use positive and negative controls as needed.
Slides	MLS Use high quality slides made from crown, borosilicate, or soda lime glass.
Coverslips	MLS Use coverslip recommended by the objective manufacturer. Most of the oil immersion objective manufacturers recommend #1.5 coverslips made out of high-quality glass, such as Thermo Scientific™ Gold Deal™ Cover Slips (Fisher Scientific™, Cat. No. 12-519-21A).
Conjugated probes for FISH, as needed	MLS
Primary or secondary antibodies, as needed[2]	MLS
Ethanol	MLS
Glycerol, fluorescent microscopy grade	MLS
Distilled water	Thermo Fisher Scientific (Cat. No. 15230147
Image-iT™ Fixation/Permeabilization Kit	Thermo Fisher Scientific (Cat. No. R37602)
To detect low abundant targets, SuperBoost™ Tyramide Signal Amplification is recommended. Learn more at **thermofisher.com/superboost**.	

[1] MLS: Fisher Scientific™ (**fisherscientific.com**) or other major laboratory supplier.

[2] To search through the vast Thermo Fisher Scientific™ primary antibody collection, visit our antibody search tool at **thermofisher.com/us/en/home/life-science/antibodies**.

Table 1 Selection guide for ProLong™ and SlowFade™ antifade mountants.

Feature	ProLong™ Glass	ProLong™ Diamond	ProLong™ Gold	SlowFade™ Glass	SlowFade™ Diamond	SlowFade™ Gold
Hard/Soft setting	Hard-setting (curing)			Soft-setting (non-curing)		
Refractive index	~1.52 after curing (same as crown glass)	~1.47 after curing	~1.47 after curing	~1.52	~1.42	~1.42
Z-stacking, 3D reconstruction/deconvolution	Yes	Not recommended		Yes	Not recommended	
Recommended objective type	Oil-immersion[1]	Glycerol-, water-, or air-corrective objectives[2]		Oil-immersion [1]	Glycerol-, water-, or air-corrective objectives[3]	
Cell/tissue thickness	0–150 µm	Best results from 0–10 µm. Imaging up to 30 µm is possible under optimized conditions.		0–500 µm	Best results from 0–10 µm. Imaging up to 30 µm is possible under optimized conditions.	
Organic dye photobleach protection[4]	+++	+++	++	++	+++	++
Fluorescent protein photobleach protection[4]	+++	+++	Not recommended	++	+++	Not recommended
Slide storage after mounting	Long term (weeks to months)			Short term (days to weeks)		

[1] Best results are obtained with oil-immersion objective. Other objective types are also compatible.

[2] Best results are obtained with glycerol-corrective objective. Other objective types are also compatible.

[3] Best results are obtained with glycerol-corrective objective. Other objective types are also compatible.

[4] 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 times with 1.58 µs dwell time per pixel. Excitation wavelength and intensity were optimized by fluorophore. On an epi-fluorescence microscope using 100-watt Hg-arc lamp, this amount of light/photon exposure will be equal to 60–90 seconds. In the table, "+++" designates when 90% of signal intensity was left, as compared to initial signal intensity. "++" designates 80–90% remaining signal intensity and "+" represents 60–80% remaining signal intensity.

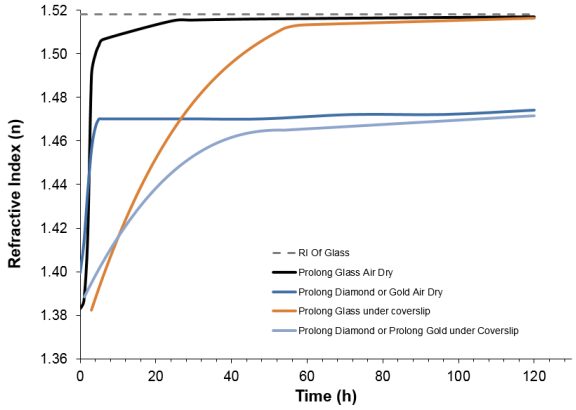

Fig. 1 ProLong™ Antifade Mountant curing time for final refractive index. The refractive indices of various ProLong™ Antifade Mounting Media were measured using a Thermo Scientific™ ABBE-3L refractometer. For this analysis, 300 µL of mountant was applied to cover the measuring prism surface. Mountants 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.

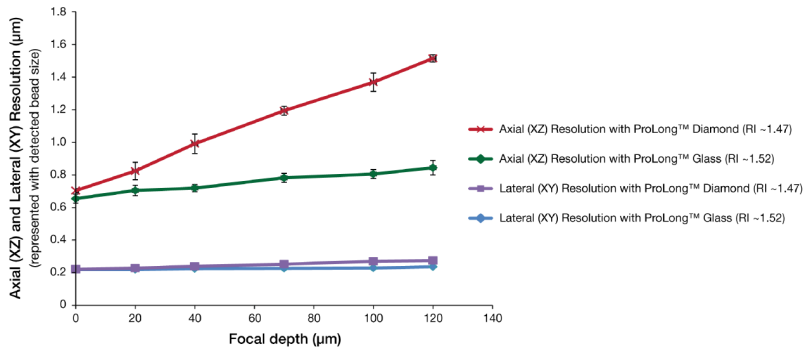

Fig. 2 Lateral and axial resolution as a point spread function of detected 170-nm microspheres. To detect the lateral and axial resolution at various focal depths, sub-resolution fluorescent yellow (Ex/Em 505 nm/515 nm) 170-nm microspheres were suspended on the surface of a glass coverslip. Same 170-nm microspheres were also mixed with 100 µL of either ProLong™ Glass (RI~1.52 after curing) or ProLong™ Diamond (RI~1.47 after curing) and suspended throughout the mountant. The mountant-microsphere mixture was spread over a 18 mm × 18 mm area on a microscope slide and was left to cure without a coverslip on it. Next day, 10 µL of glycerol was placed on top of each sample and coverslips (Zeiss™ high tolerance #1.5 170 nm ± 5 nm) were mounted on the microscope slides (a thin layer of undiluted gycerol helps the coverslip to adhere and mount on to the microscope slide). Coverslips were allowed to adhere for 1 hour before imaging. Z-stacks of individual microspheres were collected on a Zeiss™ LSM 710 confocal microscope using a Plan-Apochromat 63x/1.4 NA Oil objective, sampling at a rate of 42 nm in x, y and 100 nm in the z dimensions. Lateral (x, y) and axial (z) resolutions were calculated using the ImageJ MetroloJ plugin. Plotted data shows axial and lateral resolutions as a function of focal depth. ProLong™ Glass with a refractive index of ~1.52 maintains a higher axial resolution than mountants with a refractive index of ~1.47 across focal depts. Lateral resolution remains the same in both mountants at all focal depths tested as expected. The maximum theoretical axial resolution of the microscope is 500 nm, with 200 nm for lateral direction.

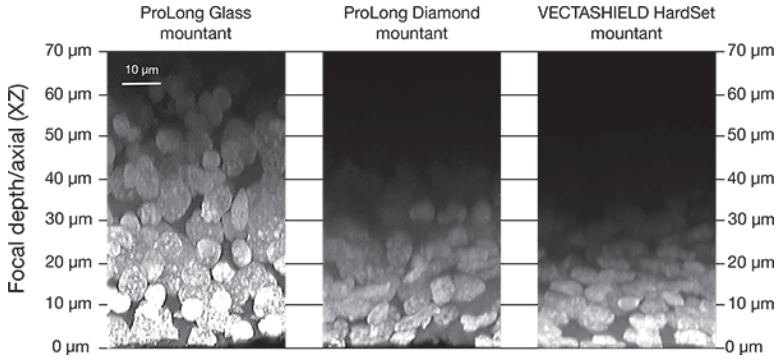

Fig. 3 ProLong™ Glass Antifade Mountant improved the depth of imaging by 40%, allowing nuclei to be imaged 70 µm into the tissue section. FFPE pig-brain sections were processed and stained with DAPI nuclear stain overnight. Stained samples were mounted with different commercial, hard-setting antifade media according to each mounting medium's recommended protocols. Tissue sections were imaged on a Zeiss™ LSM 710 confocal microscope using a Plan-Apochromat 63×/1.4 NA Oil objective sampling at a rate of 83 nm in the x and y dimensions and 100 nm in the z dimension, with a pixel size of 0.07 µm. Z-projections were generated using Zeiss™ Zen software.

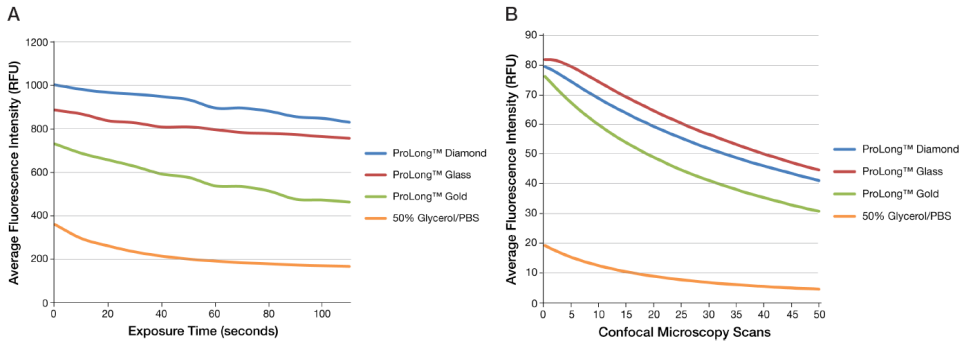

Fig. 4 FITC photobleach curves with widefield (A) and confocal (B) microscopes.

(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.

Important procedural guidelines

- **Use at room temperature.** Allow the ProLong™ Glass Antifade Mountant to warm to room temperature for 1 hour before using it to mount coverslips.
- **Do not shake.** Avoid shaking the bottle to prevent the introduction of air bubbles that can make mounting and imaging difficult.
- **Allow to cure completely to achieve refractive index of ~1.52.** ProLong™ Glass Antifade Mountant reaches its maximal refractive index of ~1.52 upon curing, as detected by a refractometer. Extra water in and around the specimen will affect the curing time and the refractive index. For specimens thicker than 30 µm, we recommend mounting "Option B" on page 4 or "Option C" on page 4 for optimal results.
- **The use of spacers is *not recommended*.** When mounting thicker specimens with ProLong™ Glass, use "Option C" on page 4.
- **For immediate viewing.** Specimens mounted in ProLong™ Glass can be viewed within 30-60 minutes after mounting. For best results, allow the mountant to cure completely.
- **For extended storage.** Following curing as described, store the mounted slides in a dry, dark container between -20°C and room temperature. Storage time is dependant upon specimen type, fluorophores used, and storage temperature. We recommend storage at -20°C for the longest storage time.
- **Not recommended for lipophilic membrane stains.** ProLong™ Glass Antifade Mountant contains glycerol, which may interfere with the use of lipophilic membrane stains such at DiI.
- 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.

Methods

Choose one of the following mounting options based on the thickness of the specimen and the allowed curing time.

Option A

For cultured monolayer cells and tissue sections with a thickness of <30 µm (Overnight to 24 hours of total curing time).

1. **Warm to room temperature:** Allow the mountant to warm to room temperature for 1 hour before mounting specimens.
2. **Apply mountant:** Remove excess liquid from the sample by gently tapping the edge of the coverslip or slide on a laboratory wipe.
   - **For coverslip-mounted specimens:** Apply 1–2 drops or 20–60 µL of the mountant directly onto a clean slide, then carefully lower a coverslip onto the mountant to avoid trapping any air bubbles.
   - **For slide-mounted specimens:** Apply 1–2 drops or 20–60 µL of the mountant directly to the specimen, then carefully lower a coverslip onto the mountant to avoid trapping any air bubbles.
3. **Allow to cure overnight:** Place the mounted sample on a flat, dry surface, and allow it to cure for 18–24 hours at room temperature in the dark.

Option B

For specimens 30–100 µm and thicker - Standard curing protocol (48 to 60 hours of total curing time)

1. **Warm to room temperature:** Allow the mountant to warm to room temperature for 1 hour before mounting specimens.
2. **Apply mountant:** Remove excess liquid from the sample by gently tapping the edge of the coverslip or slide on a laboratory wipe.
   - **For coverslip-mounted specimens:** Apply 2–3 drops or 60–100 µL of the mountant directly onto a clean slide, then carefully lower a coverslip onto the mountant to avoid trapping any air bubbles.
   - **For slide-mounted specimens:** Apply 2–3 drops or 60–100 µL of the mountant directly to the specimen, then carefully lower a coverslip onto the mountant to avoid trapping any air bubbles.
3. **Allow to cure:** Place the mounted sample on a flat, dry surface, and allow it to cure for 48 to 60 hours at room temperature in the dark.

Option C

For specimens up to 150 µm - Fast curing protocol with best refractive index and resolution (19 to 27 hours of total curing time).

1. **Warm to room temperature:** Allow the mountant to warm to room temperature for 1 hour before mounting specimens.
2. **Apply mountant:** Remove excess liquid from the sample by gently tapping the edge of the coverslip or slide on a laboratory wipe.
   - **For coverslip-mounted specimens (recommended):** Apply 2–3 drops or 60–100 µL of the mountant directly onto the specimen. Carefully tilt the coverslip back-and-forth to distribute mountant evenly over the specimen and the coverslip. You can use the edge of a pipette tip to gently assist in removing any bubbles and spreading the mountant. Proceed to Step 3. **Do not place the coverslip on a microscope slide at this point**.
   - **For slide-mounted specimens:** This method is recommended only for specimens 50 µm or less, or if you are planning to use a long working distance objective. Apply 2–3 drops or 60–100 µL of the mountant directly to the specimen. Carefully tilt the slide back-and-forth to distribute mountant evenly over the specimen and the slide. You can use the edge of a pipette tip to gently assist in removing any bubbles and spreading the mountant. Proceed to Step 3. **Do not apply a coverslip to the specimen at this point**.
3. **Allow to cure overnight:** Place the mounted sample on a flat, dry surface, and allow it to cure for 18–24 hours at room temperature in the dark. Tissue will be more transparent than before mounting. Mount should be rigid around the specimen, eliminating the need for spacers.

   Note: Specimens can be fully cured in 60 minutes in a dry 37°C incubator.

4. **Apply a thin layer of glycerol or immersion oil:** After 18–24 hours, apply 10–30 µL of glycerol or immersion oil across the top of the cured mountant and specimen.

   Note: Apply just enough glycerol to create a very thin layer across the mounted specimen to adhere the coverslip in place for the next step. The thin layer of glycerol does not seem to change the refractive index for the light path significantly while still allowing the capture of images with minimum optical refraction.
5. **Apply coverslip to microscope slide:** Apply a coverslip to the microscope slide and gently press it into place, tapping to remove bubbles as necessary. Make sure that no air bubbles exist between the mounted sample and the microscope slide. If necessary, apply additional glycerol. The coverslip can be tacked in place with paraffin and imaged immediately; however, the coverslip will begin to set into place over 1–3 hours.

Fluorescence microscopy

Note: If ProLong™ Glass Antifade Mountant with NucBlue™ (Hoechst 33342) is used, then the cellular nucleus can be imaged using a fluorescence microscope with a traditional DAPI filter set. NucBlue™ (Hoechst 33342) is a dsDNA stain with an Ex/Em of 350/461 nm.

Note: If ProLong™ Glass Antifade Mountant with SYTOX™ Deep Red Nucleic Acid Stain 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.

You can image the samples with a fluorescence microscope before the mounting medium cures. However, the refractive index, as well as the antifade effectiveness, improve following curing for 18-48 hours. When properly handled and stored as recommended, samples can be imaged with minimal photobleaching for up to 3 months.

To further impede photobleaching, minimize the exposure of fluorescently-labeled samples to light by using neutral density filters and limit exposure times and exposure intensity. Using LED light cubes from the EVOS™ microscopy system or similar tools can also be highly beneficial in reducing photobleaching and enhancing sensitivity.

ProLong™ Glass Antifade Mountant is compatible with most fluorescent microscopes and objectives, such as epi-fluorescent, wide-field, confocal, stimulated emission depletion (STED), and structured illumination microscopy (SIM). For best results, we recommend oil immersion objectives with a high numerical aperture.

Extended storage of samples

Following the curing time, the edges of the coverslip can be completely sealed with epoxy or VALAP. The sample can be 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.

Removal of 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 the SlowFade™ Glass Diamond or SlowFade™ Gold Antifade Mountants.

1. To remove mounted coverslips, place the mounted slide into a Coplin jar with phosphate buffered saline at room temperature and gently agitate overnight.
2. Once the coverslip has detached from the slide, carefully rinse the slide or coverslip with additional PBS or water to remove residual mountant.
3. Carefully note which side of the coverslip or slide contains the specimen before continuing with additional manipulation or staining of the specimen.

Appendix

Axial resolution and signal intensity

Studies show that the axial resolution of a microscopy system can be improved by matching the refractive index (RI) of mounting media, which maximizes light collection by the objective lens.[1–3] Fouquet *et al.* saw dramatic improvement in axial resolution while using a confocal microscope with high-RI media.[1]

ProLong™ Glass Antifade Mountant is a hard-setting, curing mountant that forms an optical path with minimal optical distortion, because its RI of ~1.52 (after curing) matches that of crown glass used in coverslips. When ProLong™ Glass mountant with a refractive index of ~1.52 is compared with a mounting medium with a refractive index of ~1.47, the axial resolution is improved by 32% at focal depth of 40 µm and 75% at focal depth of 100 µm. Because the optical light path is improved due to matched refractive index, the signal intensity and sensitivity of fluorophores are also improved in deep tissues. The lateral direction resolution is unchanged (Figure 1). This property makes the ProLong™ Glass Antifade Mountant an excellent mountant to capture Z-stack and 2-D images of cells and tissues at any depth from 0 µm to 100 µm.

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.

Example immunohistochemistry (IHC) protocol for 100 µm-thick FFPR rat brain section

Note: This is an example protocol. Each step can be optimized for varying experimental conditions.

1. **Deparaffinize 100 µm-thick FFPE rat brain sections in 50 mL conical tubes (Note: Sections brittle until hydrated, handle carefully!).**
   a. Xylene (2 × 15 min), 50:50 Xylene:EtOH (15 min), 100% EtOH (2 × 10 min), 95% EtOH (10 min), 85% EtOH (10 min), 75% EtOH (10 min), Water (5 min), 1X PBS (5 min)
2. **Antigen retrieval (carry out remaining protocol in 50 mL conical or 6-well plate, 1.5-2 mL volumes recommended)**
   a. 100% MeOH (5 min), 20% DMSO in MeOH (2 × 5 min), 80% MeOH in PBS (5 min), 50% MeOH in PBS (5 min)
   b. Wash with PBS at room temperature (2 incubations × 5 min).
3. **Permeabilization for antibody access**
   a. PBS/1% Triton™ X-100 at room temperature (2 incubations × 5 min)
   b. CytoVista™ Penetration Buffer (Product No. V11310): PBS/0.2% Triton™ X-100 / 0.3 M glycine / 20% DMSO at room temperature (1 incubation × 15 min)
4. **Block tissue sections**
   a. CytoVista™ Blocking Buffer (Product No. V11308): PBS/0.2% Triton™ X-100 /6% donkey serum/10% DMSO for 1 hour at room temperature (1 incubation × 1 hour)
5. **Primary antibody incubation (screen concentrations, *include no primary control*)**
   a. Formulate antibodies in CytoVista™ Antibody Dilution Buffer (Product No. V11305): PBS/0.2% Tween™ 20/10 µg/mL Heparin/3% donkey serum/5% DMSO add to tissue (1 incubation × overnight at room temperature).

      Note: For the first time for each primary, titrate the primary antibody concentration for optimal results. Too low or too high primary antibody can result in sub-optimal or no labeling. This concentration can be different than used for thin tissues.
   b. Wash with CytoVista™ Wash Buffer diluted to 1X (Product No. V11312):

      PBS/0.2% Tween™ 20/10 µg/mL Heparin (5 washes × 10 min)
6. **Secondary antibody incubation (screen concentrations)**
   a. Formulate antibodies in CytoVista™ Antibody Dilution Buffer: PBS/0.2% Tween™ 20/10 µg/mL Heparin/3% donkey serum/5% DMSO. Nuclear counter stain at 1X concentration, can combine with secondary antibody (1 incubation × overnight at room temperature).
   b. Wash with CytoVista™ Wash Buffer diluted to 1X:

      PBS/0.2% Tween™ 20/10 µg/mL Heparin (5 washes × 10 min).
   c. Rinse with water prior to mounting.
7. **Mount tissue sections with ProLong™ Glass (Product No. P36980) using fast drying technique for best results (see "Option C" on page 4**)
   a. Place labeled brain section on a coverslip.
   b. Add 2-3 drops of mountant on top of the section.
   c. Smooth out the mountant surface using a pipette, if needed. Pop any bubbles.
   d. Cure the coverslip with tissue and mountant overnight on a level surface in a dry, dark space at room temperature. Do not apply to a microscope slide at this point.

e. After the overnight incubation, examine the cured specimen. There should be remarkable difference in transparence of tissue before and after mounting (Figure 5). ProLong™ Glass should be rigid around the specimen, eliminating the need for spacers.

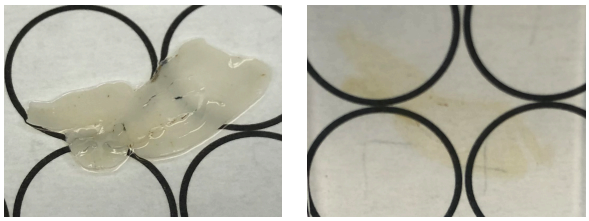

Fig. 5 Before and after mounting on ProLong™ Glass of 100 µm-thick rat brain section.

f. Place 10-30 µL of 100% glycerol on top of the mounted tissue. Even out the glycerol on the surface using a pipette tip.

g. Invert cured sample with glycerol onto a microscope slide, sandwiching the tissue between coverslip and microscope slide. Incubate for 1-3 hours until microscope slide is held rigidly in place.

h. Image tissue using a confocal microscope. A typical result is presented in Figure 6.

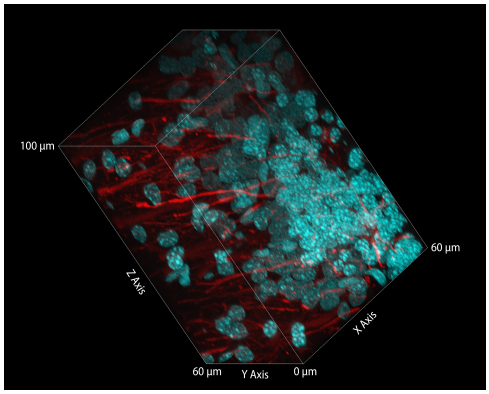

Fig. 6 FFPE rat brain sections (100 µm thick), stained for tubulin (red) with Mouse Anti-Beta3-Tubulin (Cat. No. MA1-118) and Alexa Fluor™ Plus 594 Goat Anti-Mouse (Cat. No. A-11032). Nuclei (Cyan) were stained with SYTOX™ Deep Red Nucleic Acid Stain. Slides were mounted with ProLong™ Glass Antifade Mountant (Cat. No. P36982) and imaged with a confocal microscope using 63X oil immersion objective.

Photobleach resistance of mounted fluorophores

Table 2 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 immunocytochemistry (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 microscope 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.

References

1. PLoS ONE 10(3): e0121096. doi:10.1371/journal (2015);
2. Mol Bio of Cell 26, 4075 (2015);
3. Eur Phys J H 38, 281 (2013).

Ordering information

Cat. No.	Product Name	Unit Size
P36993-5X2ML	ProLong™ Glass Antifade Mountant with SYTOX™ Deep Red	5 × 2 mL
P36993	ProLong™ Glass Antifade Mountant with SYTOX™ Deep Red	2 mL
P36994	ProLong™ Glass Antifade Mountant with SYTOX™ Deep Red	10 mL
P36980	ProLong™ Glass Hard-set Antifade Mountant	5 × 2 mL
P36982	ProLong™ Glass Hard-set Antifade Mountant	2 mL
P36984	ProLong™ Glass Hard-set Antifade Mountant	10 mL
P36981	ProLong™ Glass Hard-set Antifade Mountant with NucBlue™	5 × 2 mL
P36983	ProLong™ Glass Hard-set Antifade Mountant with NucBlue™	2 mL
P36985	ProLong™ Glass Hard-set Antifade Mountant with NucBlue™	10 mL
Related Products		
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
P36987-5X2ML	ProLong™ Gold Antifade Mountant with SYTOX™ Deep Red	5 × 2 mL
P36987	ProLong™ Gold Antifade Mountant with SYTOX™ Deep Red	2 mL
P36988	ProLong™ Gold Antifade Mountant with SYTOX™ Deep Red	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
P36990-5X2ML	ProLong™ Diamond Antifade Mountant with SYTOX™ Deep Red	5 × 2 mL
P36990	ProLong™ Diamond Antifade Mountant with SYTOX™ Deep Red	2 mL
P36991	ProLong™ Diamond Antifade Mountant with SYTOX™ Deep Red	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
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
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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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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