SlowFade™ Antifade Mountants

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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

SlowFade™ Antifade Mountants are ready-to-use mountants applied directly to fluorescently labeled cell or tissue samples on microscope slides. These reagents contain chemical components designed to improve the refractive index and protect fluorescent dyes from fading (photobleaching). These features enable sharper images and higher signal sensitivities during fluorescence microscopy. The SlowFade™ mountants are glycerol-based, non-curing mountants that allow for immediate sample viewing with no curing artifacts to affect cellular/tissue morphology.

SlowFade™ Glass Antifade Mountant features a 1.52 refractive index, which is similar to that of glass coverslips, compatible immersion oil, and oil-immersion microscope optics. This enables a refraction-free light path with minimized spherical aberration improving axial resolution by 2-to-3 fold at a 150 µm focal depth (Figure 1) as compared to mountants with either a 1.42 or 1.45 refractive index. In a biological specimen, this correction of refractive index increases optical transparency (Figure 2) resulting in sharper images at increased focal depths (Figure 3).

SlowFade™ Glass Antifade Mountant is also designed to provide excellent protection against photobleaching across the visible and near infrared spectra. It can be used with most fluorescent dyes or fluorescent proteins (e.g., GFP, RFP, mCherry) (Table 1). This is the ideal mountant for specimens with imaging depths from 0-500 µm, including cultured cells, organoids/spheroids, and thin and thick tissue sections (Figure 4).

SlowFade™ Gold Antifade Mountants and SlowFade™ Diamond Antifade Mountants all have a refractive index of 1.42 and are recommended for cultured cells or tissue sections with a thickness of less than 15 µm. SlowFade™ Diamond Antifade Mountant provides the best sensitivity and protection against photobleaching for Alexa Fluor™ dyes, classic organic dyes (FITC, TRITC, and Texas Red), and fluorescent proteins (GFP, RFP, mCherry). SlowFade™ Gold Antifade Mountant is not recommended for mounting samples containing fluorescent proteins (e.g., GFP) or classic organic dyes (FITC, TRITC, and Texas Red) . Consult Table 1 for comparative photobleaching protection between the 3 different SlowFade™ mountants.

Contents and storage

	SlowFade™ Glass Antifade Mountant	SlowFade™ Diamond Antifade Mountant	SlowFade™ Gold Antifade Mountant
Product	S36917 (1 × 2 mL)	S36967 (1 × 2 mL)	S36940 (1 × 2 mL)
	S36918 (1 × 10 mL)	S36972 (1 × 10 mL)	S36936 (1 × 10 mL)
	S36917-5X2ML (5 × 2 mL)	S36963 (5 × 2 mL)	S36937 (5 × 2 mL)
Product with DAPI	S36920 (1 × 2 mL)	S36968 (1 × 2 mL)	S36942 (1 × 2 mL)
	S36921 (1 × 10 mL)	S36973 (1 × 10 mL)	S36938 (1 × 10 mL)
	S36920-5X2ML (5 × 2 mL)	S36964 (5 × 2 mL)	S36939 (5 × 2 mL)
Recommended specimen thickness	Up to 500 µm	Up to 15 µm	
Refractive index	1.52	1.42	
Cell/Tissue types	• Tissue culture cells • Tissue sections up to 500 µm thick (FFPE and cryo-sectioned) • Organoid/Spheroids up to 500µm thick	• Tissue culture cells • Tissue sections up to 15 µm thick (FFPE and cryo-sectioned)	
Photobleach protection	Better	Best	Good
Recommended for Alexa Fluor™ fluorophores	Yes	Yes	
Recommended for classic organic dyes (e.g., FITC, TRITC, etc.)	Yes	Yes	No
Recommended for fluorescent proteins (e.g., GFP, RFP, etc.)	Yes	Yes	No
Storage[1]	Store at –15 to –20°C. Protect from light. Store in bag provided with desiccant or a similar dry environment. (Recommended)	Store at 2-8°C. Protect from light.	Store at room temperature (15-30°C). Protect from light.

[1] Product may also be stored at ≤ -20°C. When stored as directed, product is stable for a minimum of 6 months.

Important procedural guidelines

- Allow the SlowFade™ Antifade Mountants to warm to room temperature. If the vials are refrigerated or frozen, thaw for 1 hour at room temperature.
- Avoid shaking the bottle to prevent air bubbles.
- Use spacers if necessary for thick specimens.
- For extended storage after mounting and sealing the slides, store the mounted slides in a dry, dark container at –15 to –20°C.
- Storage time is dependent upon specimen type, fluorophores used, and storage temperature. For extended slide storage (months to years), mounting in ProLong™ Glass Antifade Mountant is highly recommended.
- Not recommended for lipophilic membrane stains. SlowFade™ Antifade Mountants contain glycerol, which may interfere with the use of lipophilic membrane stains such as DiI.
- For tissues 500 µm to 10 cm thick, or hard to clear tissues (e.g., heart, liver, etc.), CytoVista™ Tissue Clearing/Staining Kit (Catalog No. V11324) is highly recommended.
- For microplate imaging, including high-content imaging, CytoVista™ 3D Cell Culture Clearing/Staining Kit (Catalog No. V11325) is recommended.
- For plant tissue clearing and mounting, Image-iT™ Plant Tissue Clearing Reagent (Catalog No. V11328) is recommended.

Prepare a slide using SlowFade™ mountant

1. Warm the vial of mountant to room temperature. If frozen, allow the vial to warm at room temperature for 1 hour before using to mount coverslips.
2. Remove excess liquid from the sample by gently tapping the edge of the coverslip or slide on a laboratory wipe.

   Note: Residual liquid will interfere with mounting and must be carefully removed for best results. When dealing with multiple slides, handle one slide at a time to remove excess liquid and mount the sample.
3. Apply the mountant.
   - For coverslip-mounted specimens, apply 1-2 drops or 20-60 µL of the mountant directly onto a clean microscope slide. 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. Carefully lower a coverslip onto the mountant to avoid trapping any air bubbles.
4. Seal the edges of slide with VLAP or other sealing material.
   - Samples < 15 µm thick can be imaged immediately after mounting.
   - For samples 15 µm to 1,000 µm thick, only SlowFade™ Glass Antifade Mountant is recommended. For these samples, overnight incubation may be needed. As demonstrated in Figure 2, at the end of incubation the specimen will demonstrate a decrease in opacity.
5. Image the slides using optimized microscope settings.

   Note: DAPI stained specimens can be viewed with a DAPI common filter set. DAPI stains have excitation/emission maxima of 350/470 nm.
6. Store the mounted slides in a dry, dark container at –15 to –20°C on a flat surface.

   Note: For extended slide storage (months to years), mounting with ProLong™ Glass Antifade Mountant is recommended.

   Note: Phalloidin conjugate-stained specimen slides must be stored at –15 to –20°C immediately after imaging is complete. If stored properly, these samples can be imaged for at least 2 weeks. For extended storage, mounting in ProLong™ Glass Antifade Mountant is recommended.

Fluorescence microscopy guidelines

- Many samples can be imaged with a fluorescence microscope immediately after mounting, but for optimal resolution, incubate ≤ 15 µm-thick specimens for 30 minutes. For specimens between 15 µm and 1,000 µm overnight incubation is highly recommended.
- To further impede photobleaching, limit exposure times and intensities, and minimize the exposure of fluorescently labeled samples to light by using neutral density filters.
- Using LED light cubes from the EVOS™ microscopy system or similar tools can be highly beneficial in reducing photobleaching and enhancing sensitivity.
- SlowFade™ Antifade Mountants are compatible with most fluorescent microscopes and objectives, such as epi-fluorescent widefield, confocal, stimulated emission depletion (STED), and structured illumination microscopy (SIM). For best results, we recommend objectives closely matched to the mountant refractive index, with a high numerical aperture.

Remove mounted coverslips

1. Carefully remove the slide sealant along the edges with a sharp blade.
2. Place the mounted slide into a Coplin jar with phosphate-buffered saline (PBS) at room temperature and gently agitate for 30 minutes or until the coverslip detaches.
3. Once the coverslip has detached from the slide, carefully rinse the slide or coverslip with additional PBS or water to remove residual mountant.
4. Carefully note which side of the coverslip or slide contains the specimen before continuing with additional manipulation or staining of the specimen.

Appendix

Additional figures

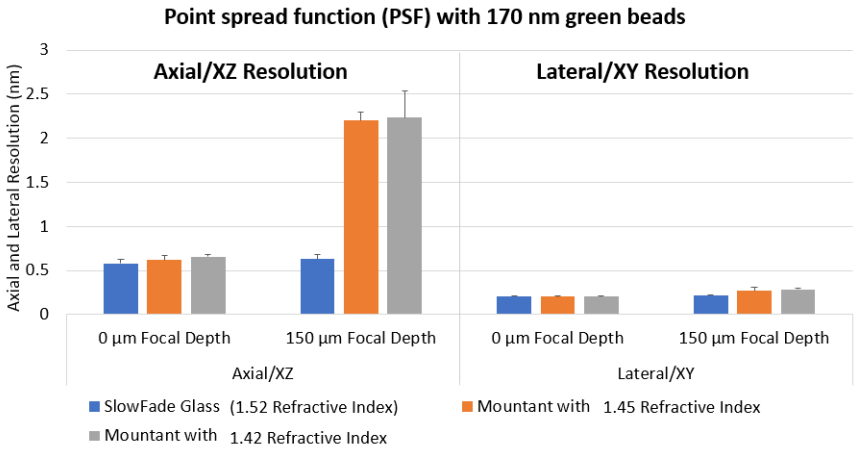

Fig. 1 Lateral and axial resolution as a point spread function of detected 170 nm microspheres. To detect the lateral and axial resolution at shallow and deep focal depths, sub-resolution fluorescent yellow (Ex/Em 505 nm/515 nm) 170-nm microspheres were absorbed onto the surface of a glass coverslip and a microscope slide. Two pieces of tape were stacked and used as spacers to position mounted coverslips (Zeiss™ high tolerance #1.5 170 nm ± 5 nm) approximately 150 µm from the microscope slide. Microspheres were mounted in SlowFade™ Glass (RI ~1.52) or SlowFade™ Diamond (RI ~1.42) or VECTASHIELD™ (R1 ~1.45) and coverslips were adhered to the microscope slides with paraffin. Z-stacks of individual microspheres (five at each focal depth) 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 for microspheres absorbed to the coverslip (0 µm) and microscope slide (150 µm). SlowFade™ Glass with a refractive index of ~1.52 maintains a higher axial resolution than mountants of 1.42 and 1.45 refractive index at 150 µm focal dept. Lateral resolution remains the same in all 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. 2 Clearing of 1-mm mouse brain section by refractive index matching with SlowFade™ Glass at time intervals of 0, 16, and 48 hours.

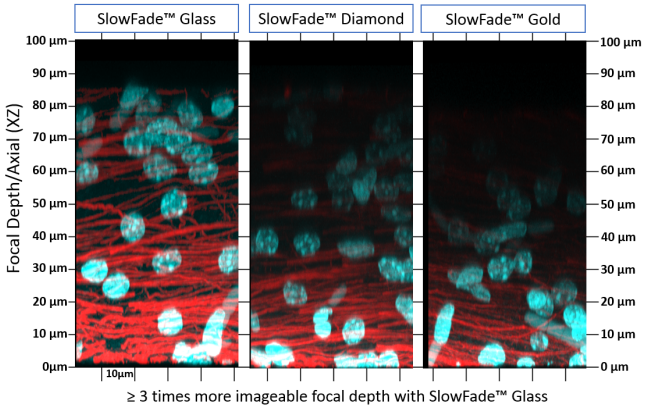

Fig. 3 Improved focal depth in 100 µm-thick brain tissue sections with SlowFade™ Glass Antifade Mounting Media refractive index matched to 1.52. Cryo-preserved 100 µm-thick rat brain sections were stained for GFAP (red) with Rabbit Anti-GFAP (Cat. No. OPA1-06100) and Alexa Fluor™ Plus 594 Goat Anti-Rabbit (Cat. No. A-32740) overnight. Nuclei (cyan) were stained with DAPI nuclear stain (Cat. No. D1306). Stained samples were mounted with SlowFade™ Glass (Cat. No. S36917) , SlowFade™ Diamond (Cat. No. S36967), or SlowFade™ Gold (Cat. No. S36940) non-curing Antifade Mounting Media. 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 71 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.

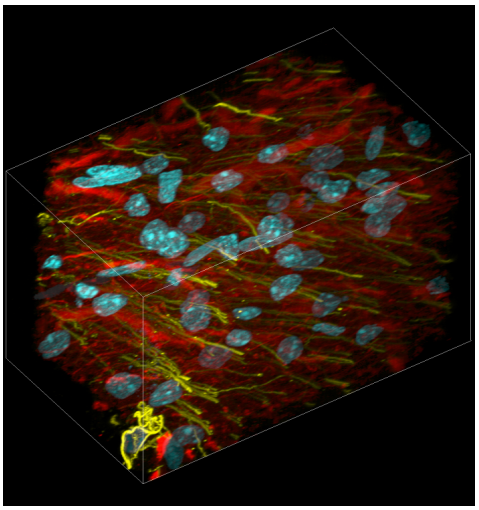

Fig. 4 Deep tissue imaging with non-curing, refractive index matched SlowFade™ Glass. Cryo-preserved rat brain sections (100 µm thick), stained for tubulin (red) with Mouse Anti-Beta3-Tubulin (Cat. No. MA1-118) and GFAP (yellow) Rabbit Anti-GFAP (Cat. No. OPA1-06100). Targets were detected with Alexa Fluor™ Plus 594 Goat Anti-Mouse (Cat. No. A-11032) and Alexa Fluor™ Plus 647 Goat Anti-Rabbit (Cat. No. A-32733) dyes. Nuclei (cyan) were stained with DAPI (Cat. No. D1306). Slides were mounted with non-curing SlowFade™ Glass Antifade Mountant (Cat. No. S36917) and imaged with a Zeiss™ LSM 710 confocal microscope using a Plan-Apochromat 63×/1.4 NA Oil immersion objective at a rate of 71 nm in the x and y dimensions and 110 nm in the z dimension, with a pixel size of 0.07 µm. Z-projections were generated using Zeiss™ Zen software.

Example immunohistochemistry (IHC) protocol for 100 µm-thick cryo-preserved rat brain sections

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

1. **Thaw 100 µm-thick cryo-preserved rat brain sections in PBS in 50 mL conical tubes.**
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. **Mounting**
   a. Place immuno-labeled brain section on a microscope slide ensuring that the specimen is flat.
   b. Using a laboratory wipe, carefully absorb all residual liquid from the sample. This will optimize sample transparency and can shorten absorption time.
   c. Add 2-3 drops of SlowFade™ Glass Antifade Mountant (e.g. Product No. S36916), to tissue section.
   d. Lower the coverslip slowly onto the specimen, one side first, while popping any bubbles.
   e. Seal the slide with VLAP, paraffin, or another sealant.
   f. Incubate the slide at room temperature overnight, on a dark, dry, flat surface.
8. **Imaging**
   a. Image tissue using a confocal microscope. A typical result is presented in Figure 4.

Photobleach resistance of mounted fluorophores

Table 1 Photobleach resistance for various fluorophores when mounted using SlowFade™ Antifade Mountants.

Fluorophore	Ex/Em (nm)	Resistance to Photobleaching[1]		
		SlowFade™ Glass	SlowFade™ Diamond	SlowFade™ Gold
Hoechst™ 33342	350/461	+++	+++	+++
DAPI	345/455	+++	+++	+++
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	++	++	Not recommended
Alexa Fluor™ 568	578/603	+++	+++	+++
Texas Red™	595/615	+++	+++	+++
Alexa Fluor™ 594	590/617	+++	+++	+++
Alexa Fluor™ Plus 594	590/617	+++	+++	+++
TO-PRO™-3	642/661	++	+++	++
Alexa Fluor™ 647	652/668	+++	+++	+++
Alexa Fluor™ Plus 647	652/688	+++	+++	+++
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 3 fields of view were scanned 15 times with a 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: +++ = 80% or more of signal intensity was left as compared to initial signal intensity. ++ = 65-80% remaining signal intensity. + = 50-65% remaining signal intensity. Not Recommended = 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

Catalog No.	Product	Size
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
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
S36937	SlowFade™ Gold Antifade Mountant	5 × 2 mL
S36940	SlowFade™ Gold Antifade Mountant	2 mL
S36936	SlowFade™ Gold Antifade Mountant	10 mL
S36939	SlowFade™ Gold Antifade Mountant with DAPI	5 × 2 mL
S36942	SlowFade™ Gold Antifade Mountant with DAPI	2 mL
S36938	SlowFade™ Gold Antifade Mountant with DAPI	10 mL
Tissue and 3D Cell Culture Clearing Mountants		
V11324	CytoVista™ Tissue Clearing/Staining Kit	1 kit
V11325	CytoVista™ 3D Cell Culture Clearing/Staining Kit	1 kit
V11328	Image-iT™ Plant Tissue Clearing Reagent	30 mL
V11331	Image-iT™ Plant Tissue Hard-Set Mountant	30 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
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

Limited product warranty

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