

Product Information

EverBrite TrueBlack® Hardset Mounting Medium

Product List

Catalog no.	Product	Unit size
23017-T	EverBrite TrueBlack® Hardset Mounting Medium	2 mL
23017		10 mL
23018-T	EverBrite TrueBlack® Hardset Mounting Medium with DAPI	2 mL
23018		10 mL
23019-T	EverBrite TrueBlack® Hardset Mounting Medium with NucSpot® 640	2 mL
23019		10 mL
23022-T	EverBrite TrueBlack® Hardset Mounting Medium with NucSpot® 680	2 mL
23022		10 mL

Storage and Handling

Store at 4°C and protect from light. Product is stable at least for 6 months from the date it is received. Warm to room temperature and mix well by gently swirling or inverting bottle before use; avoid vortexing or rapid shaking, which may introduce air bubbles. Tightly cap bottle after each use.

Spectral Properties

For media with nuclear counterstains only:

DAPI: Ex/Em 358/461 nm (with DNA)

NucSpot® 640: Ex/Em 649/668 nm (with DNA)*

NucSpot® 680: Ex/Em 685/708 (with DNA)*

*NucSpot® dyes also show dim blue fluorescence with the DAPI filter set, and should be tested for suitability before using with blue probes.

Product Description

This product is a unique hardset mounting medium for combined antifade protection, coverslip sealing, and lipofuscin quenching. Lipofuscin consists of granules of oxidized proteins and lipids that accumulate in aging cells. It is a source of bright, broad-spectrum background that can make specific imaging of tissues like human or aged animal brain or retina virtually impossible unless it is quenched. This mounting medium contains TrueBlack® quenchers that are specifically designed to mask lipofuscin autofluorescence while preserving specific staining. TrueBlack® quenchers may also reduce autofluorescence from other sources, such as collagen, elastin, and red blood cells.

EverBrite TrueBlack® offers the same protection against photobleaching as our original EverBrite™ Hardset (see Related Products). Unlike VECTASHIELD® mounting medium, EverBrite™ is compatible with cyanine-based fluorophores, including Cy® dyes and Alexa Fluor® 647, and is optimally formulated for use with Biotium's CF® dyes. The medium cures to form a hard seal with the coverslip that does not require additional sealing. It has a refractive index of 1.38 before hardening, which increases to 1.42 after 24 hours of curing, and 1.46 after four days of curing, after which it remains constant. After curing completely, the refractive index is well-matched to that of coverslip glass and immersion oil (1.5).

The medium is available with or without the commonly used blue nuclear stain DAPI, or with Biotium's novel NucSpot® nuclear stains. NucSpot® 640 is a far-red DNA binding dye for the Cy®5 channel, while NucSpot® 680 is a far-red/near-IR dye for the Cy®5.5 channel. Using a far-red nuclear stain can avoid problems of cross-talk and photoconversion from DAPI. However, NucSpot® 640 also has dim blue fluorescence in the DAPI channel, and may not be suitable for imaging with blue probes, especially by epifluorescence microscopy.

See Related Products for our original TrueBlack® quenchers and other mounting medium formulations.

EverBrite TrueBlack® Hardset Mounting Medium

PSF006

Protocol for Mounting Coverslips

Note: EverBrite TrueBlack® may reduce specific fluorescence signal. Antibody or probe concentration may require optimization for use with the mounting medium.

Note: EverBrite TrueBlack® Hardset Mounting Medium is designed for mounting thin tissue sections (5-15 µm) or cells cultured on coverslips without a dehydration step. It may not be suitable for mounting thick sections or non-biological specimens because bubbles may form during curing.

Note: For best results, cells or tissue sections should be permeabilized for nuclear staining with DAPI or NucSpot® dyes. Nuclear staining of non-permeabilized cells requires longer incubation times.

- At the end of your staining protocol, remove excess buffer by tapping the slide and using a lab wipe to wick away large drops of buffer. The specimen does not need to be perfectly dry.
- To mount a 22 mm² coverslip, place 2 drops (~50 µL) of EverBrite TrueBlack® Hardset medium onto the specimen. Place the coverslip on top of the medium and allow it to spread under the entire surface of the coverslip. Larger specimens/coverslips may require using more medium.
- Carefully press straight down on the surface of the coverslip with a lab wipe to blot up excess medium and remove air bubbles, taking care not to slide the coverslip from side to side.
- To cure the mounting medium to form a permanent hard seal, incubate slides overnight at room temperature on a flat surface protected from light. The refractive index will continue to increase as the medium cures.

Note: The mounting medium will harden enough to immobilize the coverslip after about 30 minutes, but care should be taken when handling coverslips before the medium is completely hardened. If you wish to image samples immediately after mounting, we recommend securing the coverslip in place by sealing one corner with nail polish or CoverGrip™ Coverslip Sealant and allowing the sealant to dry before imaging. If immersion oil is used for imaging, gently wipe it off with a lab wipe while taking care not to move the coverslip before allowing the medium to cure overnight.

Note: Lipofuscin quenching may be incomplete if slides are imaged shortly after mounting. Quenching continues to improve during curing at room temperature overnight.

- Mounted and cured slides can be stored at 4°C or -20°C, protected from light, for a year or longer.

Removing EverBrite TrueBlack® Hardset Medium

EverBrite TrueBlack® Hardset is designed to form a permanent hard seal between the slide and the coverslip. However, if necessary, coverslips can be removed after curing by soaking the slide in PBS or similar buffer in a slide staining jar. Soak the slides for 2.5-3 hours with gentle agitation to gently slide the coverslip off (do not pry the coverslip upwards), dipping the slide frequently in buffer to prevent friction between the coverslip and specimen. After the coverslip is removed, wash the slide thoroughly to remove any remaining mounting medium. To remove TrueBlack® quencher from tissue sections, rinse several times in PBS + 0.1% Triton® X-100, followed by rinsing in PBS.

Note: Washing with buffer will not remove nuclear staining with DAPI or NucSpot® dyes.

Related Products

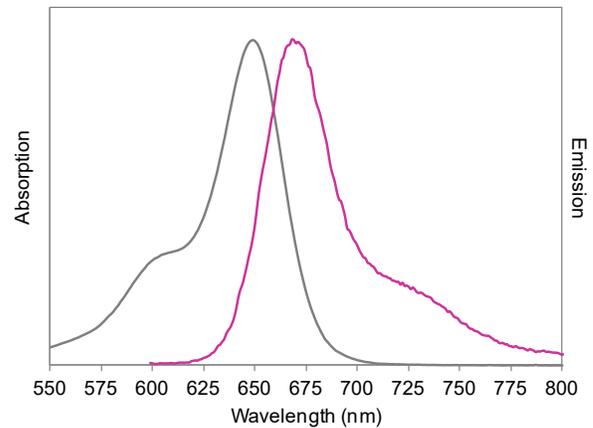
Catalog number	Product
23007	TrueBlack® Lipofuscin Autofluorescence Quencher, 20X in DMF
23015	TrueBlack® Plus Lipofuscin Autofluorescence Quencher, 40X in DMSO
23001	EverBrite™ Mounting Medium
23002	EverBrite™ Mounting Medium with DAPI
23015	EverBrite™ Mounting Medium with NucSpot® 640
23020	EverBrite™ Mounting Medium with NucSpot® 680
23008	Drop-n-Stain EverBrite™ Mounting Medium
23009	Drop-n-Stain EverBrite™ Mounting Medium with DAPI
23003	EverBrite™ Hardset Mounting Medium
23004	EverBrite™ Hardset Mounting Medium with DAPI
23016	EverBrite™ Hardset Mounting Medium with NucSpot® 640
23021	EverBrite™ Hardset Mounting Medium with NucSpot® 680
23005	CoverGrip™ Coverslip Sealant
23012	TrueBlack® IF Background Suppressor System (Permeabilizing)
40061	RedDot™2 Far Red Nuclear Counterstain, 200X in DMSO
40081	NucSpot® Live 488 Nuclear Stain
40082	NucSpot® Live 650 Nuclear Stain
40083	NucSpot® 470 Nuclear Stain
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
22005	Mini Super ^{HT} Pap Pen 2.5 mm tip, ~400 uses
22006	Super ^{HT} Pap Pen 4 mm tip, ~800 uses
22016	Permeabilization Buffer
22017	Permeabilization and Blocking Buffer
22010	10% Fish Gelatin Blocking Buffer
22011	Fish Gelatin Powder
22014	30% Bovine Serum Albumin Solution
22002	Tween®-20

Please visit our website at www.biotium.com for information on our life science research products, including fluorescent CF® dye labeled antibody, lectin, and phalloidin conjugates, Mix-n-Stain™ Antibody Labeling Kits, tyramides and tyramide amplification kits, and other fluorescent probes and accessories for cell biology research.

Cy Dye is a registered trademark of GE Healthcare; Alexa Fluor is a registered trademark of Thermo Fisher Scientific; VECTASHIELD is a registered trademark of Vector Laboratories; Triton is a registered trademark of The Dow Chemical Company; Tween is a registered trademark of Croda International LLC.

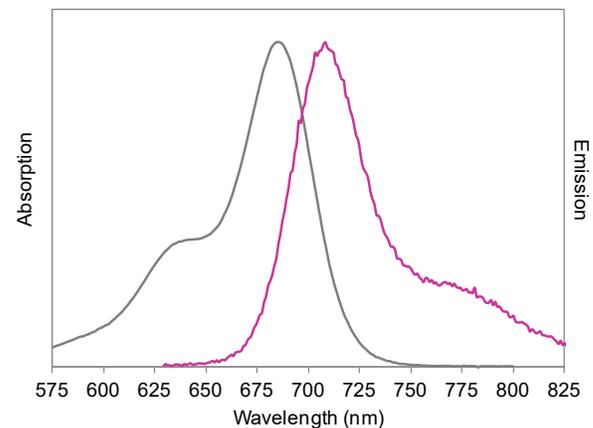
Materials from Biotium are sold for research use only, and are not intended for food, drug, household, or cosmetic use.

NucSpot® 640 Absorption and Emission



Absorption and emission spectra of NucSpot® 640 with DNA.

NucSpot® 680 Absorption and Emission



Absorption and emission spectra of NucSpot® 680 with DNA.