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Product Information

TrueBlack™ Lipofuscin Autofluorescence Quencher, 20X in DMF

Catalog Number: 23007

Unit Size: 1 mL, sufficient to treat ~100-200 tissue sections

Materials required but not supplied: 70% ethanol

Storage and Handling

Store at room temperature. Protect from light during long term storage. Product is stable for at least 12 months from date of receipt when stored as recommended.

Caution: dimethyl formamide (DMF) is hazardous, download the material safety data sheet (MSDS) for this product at www.biotium.com for more information. No information is available on the safety of TrueBlack dye. Handle the dye solution using universal laboratory precautions and dispose as hazardous waste according to your local regulations.

Product Description

Lipofuscin consists of autofluorescent granules of oxidized proteins and lipids that build up in the lysosomes of cells as a consequence of aging (1). Lipofuscin granules fluoresce brightly in all channels used for fluorescence microscopy, and accumulate in a wide variety of different cell and tissue types with age. Consequently, imaging of specific immunofluorescence signal in adult human tissues or aged animal tissues can be virtually impossible unless methods are employed to quench or mask lipofuscin fluorescence.

Traditionally, Sudan Black B has been used to quench lipofuscin autofluorescence by incubating tissue sections with the dye after immunofluorescence staining (2). However, while it masks the autofluorescence from lipofuscin, Sudan Black B also introduces uniform non-specific background fluorescence in the red and far-red channels, limiting the use of fluorescent dyes in those wavelengths (3). Now Biotium has developed TrueBlack™ as a superior alternative to Sudan Black B for elimination of lipofuscin fluorescence with minimal background fluorescence. TrueBlack treatment of immunostained tissues is rapid, simple, and has minimal effect on signal from fluorescent antibodies or nuclear counterstains, thus preserving the signal-to-noise ratio of the immunostaining.

References

1. Hohn, A. and Grune, T. Redox Biol 1(1): 140, 2013.
2. Schnell, S.A., Staines, W.A., and Wessendorf, M.W. J Histochem Cytochem 47(6): 719, 1999.
3. Romijn, H.J., van Uum, J.F.M., Breedijk, I., Emmering, J., Radu, I., and Pool, C.W. J Histochem Cytochem 47(2): 229, 1999.

Lipofuscin autofluorescence quenching protocol

The following protocol is intended for researchers with basic knowledge of immunohistochemistry techniques.

1. Perform immunofluorescence staining of tissue sections using validated antibodies according to the recommended protocol for your antigen of interest.

   Note: Staining with fluorescent nuclear counterstains such as DAPI can be performed either before TrueBlack quenching (for example, during or after secondary antibody incubation) or after TrueBlack quenching (by using a fluorescence mounting medium containing DAPI, for example).

2. Just before use, dilute 20X TrueBlack to 1X in 70% ethanol. For example, add 50 uL 20X TrueBlack to 1 mL 70% ethanol. Vortex to mix well. Prepare 100-200 uL of 1X TrueBlack for each tissue section to be treated.

3. After the final wash step of your immunofluorescence staining protocol, remove slides from the wash buffer. Tap slides to remove excess wash buffer and carefully wick away as much excess buffer as possible from around the sections using a Kimwipe.

   Important: do not allow sections to dry out, because this could destroy immunofluorescence signal. A small amount of buffer on or around the tissue section is ok. Perform TrueBlack treatment on a small number of slides at a time to make sure the sections do not dry out during handling.

4. Place slides on a level surface (for example, in a humidified slide chamber used for antibody incubations). Quickly apply a generous amount of 1X TrueBlack in 70% ethanol to completely cover the tissue sections (100-200 uL per section).

5. Leave the 1X TrueBlack solution on the sections for 30 seconds.

   Note: the TrueBlack solution can be left on the sections longer (a few minutes) but take care that the solution doesn't evaporate and cause the sections to dry out.

6. Transfer the slides to a staining jar and rinse three times with PBS.

7. Coverslip the slides using a fluorescence antifade mounting medium such as Biotium's EverBrite™ mounting medium.

   Note: TrueBlack is not compatible with organic-based mounting media like DPX.

Related Products

Cat.#	Product Name	Unit Size
40061-T	RedDot™2 Far Red Nuclear Counterstain, 200X in DMSO, Trial Size (15-20 tests)	25 uL
40043	DAPI in H_2O, 10 mg/mL	10 mL
23001	EverBrite™ Mounting Medium	10 mL
23002	EverBrite™ Mounting Medium with DAPI	10 mL
23003	EverBrite™ Hardset Mounting Medium	10 mL
23004	EverBrite™ Hardset Mounting Medium with DAPI	10 mL
23005	CoverGrip™ Coverslip Sealant	15 mL
22005	Mini Super^{HT} Pap Pen 2.5 mm tip, ~400 uses	1 pen
22006	Super^{HT} Pap Pen 4 mm tip, ~800 uses	1 pen
22015	Fixation Buffer	100 mL
22016	Permeabilization Buffer	100 mL
22017	Permeabilization and Blocking Buffer	100 mL
22010	10% Fish Gelatin Blocking Buffer	100 mL
22011	Fish Gelatin Powder	2 x 50 g
22014	30% Bovine Serum Albumin Solution	100 mL
22002	Tween®-20	50 mL

Please visit our website at www.biotium.com for information on our life science research products, including fluorescent CF™dye secondary antibodies and other conjugates, antibody labeling kits, apoptosis reagents, fluorescent probes, and kits and accessories for cell biology research.