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 some 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 autofluorescence in tissues such as human brain (4) and retina (5) with minimal background fluorescence.

TrueBlack also reduces autofluorescence from other sources, such as collagen, elastin, red blood cells, and general background fluorescence. It is not as effective at quenching these sources of autofluorescence as it is for lipofuscin, but it can improve background in a variety of human and non-human tissue types.

TrueBlack treatment of tissue sections can be performed before or after immunostaining. It is rapid, simple, and has minimal effect on signal from fluorescent antibodies or nuclear counterstains.

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.
4. Fosso M.Y., McCarty K., Head E., Garneau-Tsodikova S., and LeVine H. 3rd. ACS Chem Neurosci DOI: 10.1021/acschemneuro.5b00266, 2015.
5. Chan T., Zhu L., Madigan M.C., Wang K., Shen W., Gillies M.C., Zhou F. Br J Pharmacol.172(9): 2343, 2015.

Protocols

The following protocols are intended for researchers with basic knowledge of immunohistochemistry techniques.

Protocol 1: Pre-treatment with TrueBlack

This protocol is preferred because it has negligible effect on the signal of fluorescent antibodies and stains. However, buffers containing detergent cannot be used in any the steps after TrueBlack treatment, because detergents will remove TrueBlack from the tissue. Detergent permeabilization can be performed before TrueBlack treatment, but if you need to include detergents during subsequent staining steps, use Protocol 2.

1.1 Perform fixation, deparaffinization, and/or antigen retrieval of tissue sections as required according to your standard protocols.

1.2 Permeabilize sections with detergent, if required. Wash with PBS.

1.3 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.

1.4 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®.

Note: do not allow sections to dry out, because this could affect the quality of fluorescence staining. It's okay to leave a small amount of buffer on the section.

1.5 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).

Note: Perform TrueBlack treatment on a small number of slides at a time to make sure the sections do not dry out during handling.

1.6 Leave the 1X TrueBlack solution on the sections for 30 seconds. Longer incubation times of a few minutes are fine as long as sections don't dry out.

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

1.8 Perform immunofluorescence staining with validated antibodies according to the recommended protocol for your antigen of interest.

Note: Do not use buffers containing detergents for blocking, antibody incubation, or washing. If detergents are required during these steps, use the post-treatment protocol.

1.9 Coverslip the slides using any aqueous-based fluorescence antifade mounting medium, such as Biotium's EverBrite™ mounting medium.

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

Protocol 2: Post-treatment with TrueBlack

Note: treating with TrueBlack after immunostaining may result in lower fluorescence signal from antibodies or nuclear stains.

2.1 Perform immunostaining according to your standard protocol. Nuclear stains can be added either before or after TrueBlack treatment.

2.2 Prepare 1X TrueBlack in 70% ethanol as described in step 1.3 above.

2.3 After the final step of your staining protocol, treat sections with 1X TrueBlack as described in step 1.4-1.7 above.

2.4 Coverslip the slides using any aqueous-based fluorescence antifade mounting medium, such as Biotium's EverBrite™ mounting medium.

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

Related Products

Catalog number	Product
40061-T	RedDot™2 Far Red Nuclear Counterstain, 200X in DMSO
40043	DAPI in H_2O, 10 mg/mL
23001	EverBrite™ Mounting Medium
23002	EverBrite™ Mounting Medium with DAPI
23003	EverBrite™ Hardset Mounting Medium
23004	EverBrite™ Hardset Mounting Medium with DAPI
23005	CoverGrip™ Coverslip Sealant
22005	Mini Super^{HT} Pap Pen 2.5 mm tip, ~400 uses
22006	Super^{HT} Pap Pen 4 mm tip, ~800 uses
80027	PathoGreen™ Histofluorescent Stain
22015	Fixation Buffer
22016	Permeabilization Buffer
22017	Permeabilization and Blocking Buffer
22010	10X Fish Gelatin Blocking Agent
22011	Fish Gelatin Powder
22014	30% Bovine Serum Albumin Solution
22002	Tween®-20

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