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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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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.

TrueBlack® is intensely colored and will stain clothing and plastics. Always centrifuge the vial before opening to collect solution out of the cap into the bottom of the vial. To clean spills, immediately wipe the quencher from surfaces using 70% ethanol.

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). 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 is rapid, simple, and has minimal effect on signal from fluorescent antibodies or nuclear counterstains. TrueBlack® treatment of tissue sections can be performed before or after immunostaining. Biotium also carries TrueBlack® Plus for lipofuscin autofluorescence quenching in aqueous buffer.

TrueBlack® has been validated in many publications. Please visit biotium.com to download a list of references.

References

1) Redox Biol 1(1), 140 (2013); 2) J Histochem Cytochem 47(6), 719 (1999); 3) J Histochem Cytochem 47(2): 229 (1999); 4) ACS Chem Neurosci 7(2), 171(2016); 5) Br J Pharmacol.172(9): 2343 (2015).

General Considerations for Treatment

The following are basic considerations for treatment with TrueBlack® Lipofuscin Autofluorescence Quencher. The protocols are intended for researchers with basic knowledge of immunohistochemistry techniques.

- The TrueBlack® lipofuscin quencher is hydrophobic in nature. Certain experimental conditions can cause the quencher to leave precipitates or clumps on the treated sample which can interfere with imaging. We recommend heating the vial of the stock solution of TrueBlack®, 20X in DMF to 70ºC for 5 minutes prior to diluting it in 70% ethanol to avoid this.
- The pre-treatment 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 or TrueBlack® IF Background Suppresor System during subsequent staining steps, use the post treatment protocol.
- TrueBlack® treatment has been validated with commonly used nuclear stains and fluorescent antibodies, but has been shown to be incompatible with fluorescent bungarotoxin staining. Other non-antibody ligands or probes should be tested for compatibility with TrueBlack® pre-treatment or post-staining.
- Quenching solution works best when made just before use however, it is possible to store TrueBlack® as a 1X solution in 70% ethanol. Inspect the solution, and do not use if precipitate is visible.
- Perform TrueBlack® treatment on a small number of slides at a time to make sure the sections do not dry out during handling.
- Use an aqueous-based fluorescence antifade mounting medium, such as Biotium's EverBrite™ Mounting Medium. TrueBlack® is not compatible with organic-based mountants like Permount™ or DPX.

Protocol 1: Pre-treatment with TrueBlack®

1. Perform fixation, deparaffinization, and/or antigen retrieval of tissue sections as required according to your standard protocols.
2. Permeabilize sections with detergent, if required. Wash with PBS.
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.
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.
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.
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.
7. Transfer the slides to a staining jar and rinse three times with PBS.

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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.

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

Protocol 2: Post-treatment with TrueBlack®

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

1. Perform immunostaining according to your standard protocol. Nuclear stains can be added either before or after TrueBlack® treatment.
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 step of your 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®.

   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.

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).

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

5. 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.
6. Transfer the slides to a staining jar and rinse three times with PBS.
7. Coverslip the slides using any aqueous-based fluorescence antifade mounting medium, such as Biotium's EverBrite™ Mounting Medium.

Related Products

Catalog number	Product
23014	TrueBlack® Plus Lipofuscin Autofluorescence Quencher, 40X in DMSO
23012	TrueBlack® IF Background Suppressor System (Permeabilizing)
23013	TrueBlack® WB Blocking Buffer Kit
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
23008	Drop-n-Stain EverBrite™ Mounting Medium
23009	Drop-n-Stain EverBrite™ 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

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