Product Information

TrueBlack® Lipofuscin Autofluorescence Quencher

Product List

Cat. No.	Product	Unit Size
23007	TrueBlack® Lipofuscin Autofluorescence Quencher, 20X in DMF	1 mL[1]
23011	TrueBlack® Lipofuscin Autofluorescence Quencher, 30X in DMSO	1 mL[2]

[1]1 mL is sufficient to treat ~100-200 tissue sections
[2]1 mL is sufficient to treat ~150-300 tissue sections

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: Dimethylformamide (DMF) is hazardous. Download the safety data sheet (SDS) for this product on the product page for more information. No information is available on the safety of TrueBlack®. Handle the quencher 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 quencher 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 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 autofluorescence. 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. TrueBlack® Lipofuscin Autofluorescence Quencher is available in the original formulation at 20X in DMF, and a newer formulation at 30X in DMSO, which is a less toxic solvent than DMF. Biotium also offers TrueBlack® Plus 40X in DMSO for lipofuscin quenching in aqueous buffer instead of 70% ethanol, and EverBrite™ TrueBlack® Hardset Mounting Medium for one-step mounting and quenching of thin tissue sections on slides (see Related Products).

TrueBlack® Lipofuscin Autofluorescence Quencher has been validated in many publications. Please visit www.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 a basic knowledge of immunohistochemistry techniques.

- The TrueBlack® Lipofuscin Autofluorescence 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® to 70°C for 5 minutes prior to diluting it in 70% ethanol to avoid this.
- Protocol 1 for pre-treatment with TrueBlack® before antibody staining is preferred, because it has a negligible effect on the signal of fluorescent antibodies and stains. However, if you need to use detergent during antibody staining or washing steps, then you must use Protocol 2 for post-treatment with TrueBlack® after staining (see next note).
- Buffers containing detergent cannot be used in any steps after TrueBlack® treatment, because detergents will remove TrueBlack® from the tissue. Detergent permeabilization can be performed before TrueBlack® treatment.
- 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® before staining or after staining.
- TrueBlack® works best when diluted to 1X immediately 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 (see Related Products). TrueBlack® is not compatible with organic-based mountants like Permount™ or DPX.

Materials required but not provided

- 70% ethanol

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 TrueBlack® to 1X in 70% ethanol and vortex to mix. Prepare 100-200 uL of 1X TrueBlack® for each tissue section to be treated (volumes may be scaled as needed):
   a. For 20X in DMF (Cat. No. 23007): Add 50 uL of 20X TrueBlack® to 1 mL of 70% ethanol.
   b. For 30X in DMSO (Cat. No. 23011): Add 33 uL of 30X TrueBlack® to 1 mL of 70% ethanol.
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).
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 do not dry out.
7. Transfer the slides to a staining jar and rinse three times with PBS.
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 after staining protocol.
9. Coverslip the slides using any aqueous-based fluorescence antifade mounting medium, such as Biotium's EverBrite™ Mounting Medium (see Related Products).

Protocol 2: Post-treatment with TrueBlack®

1. Perform immunostaining according to your standard protocol. Nuclear stains can be added either before or after TrueBlack® treatment.
2. Just before use, dilute TrueBlack® to 1X in 70% ethanol and vortex to mix. Prepare 100-200 uL of 1X TrueBlack® for each tissue section to be treated (volumes may be scaled as needed):
   a. For 20X in DMF (Cat. No. 23007): Add 50 uL of 20X TrueBlack® to 1 mL of 70% ethanol.
   b. For 30X in DMSO (Cat. No. 23011): Add 33 uL of 30X TrueBlack® to 1 mL of 70% ethanol.
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).
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 do not 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 (see Related Products).

Related Products

Cat. No.	Product
23014	TrueBlack® Plus Lipofuscin Autofluorescence Quencher, 40X in DMSO
23012	TrueBlack® IF Background Suppressor System (Permeabilizing)
23017	EverBrite™ TrueBlack® Hardset Mounting Medium
23018	EverBrite™ TrueBlack® Hardset Mounting Medium with DAPI
23019	EverBrite™ TrueBlack® Hardset Mounting Medium with NucSpot® 640
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
41033-41038	NucSpot® Nuclear Stains
22030	AntiFix™ Universal Antigen Retrieval Buffer, 10X
23013	TrueBlack® WB Blocking Buffer Kit
40061	RedDot™2 Far Red Nuclear Counterstain, 200X in DMSO
40043	DAPI in H_2O, 10 mg/mL
23005	CoverGrip™ Coverslip Sealant
23023, 23024	Super^{HT} PAP Pen 2.0
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 primary antibodies, fluorescent CF® Dye conjugates, Mix-n-Stain™ antibody labeling kits, apoptosis reagents, fluorescent probes, and other reagents for immunofluorescence microscopy.