Product Information

TrueBlack® Plus Lipofuscin Autofluorescence Quencher, 40X in DMSO

Catalog Number and Unit Size:
23014-T 50 uL
23014 500 uL

Storage and Handling
Store at room temperature. Do not refrigerate or freeze. Product is stable for at least 12 months from date of receipt when stored as recommended. If product has been at low temperature, heat to 37°C for 15 minutes and vortex to ensure it is fully solubilized.

No information is available on the safety of this product. Handle the solution using universal laboratory precautions and dispose as hazardous waste according to your local regulations. TrueBlack® Plus 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
TrueBlack® Plus is a next-generation lipofuscin quencher developed by Biotium chemists. TrueBlack® Plus is water-soluble, so quenching can be performed in PBS instead of ethanol. It greatly reduces lipofuscin autofluorescence with minimal far-red background.

Autofluorescence is a major source of non-specific background fluorescence in tissue sections and some primary cell types. Sources of autofluorescence include aldehyde fixatives, tissue components with endogenous fluorescence (including extracellular matrix proteins, red blood cells, and macrophages), and lipofuscin, which consists of highly autofluorescent granules of oxidized proteins and lipids that build up in the lysosomes of cells with age. While usually brightest in the blue and green wavelengths, autofluorescence has broad spectrum fluorescence that can make detection of specific fluorescence signal in tissues virtually impossible unless it is quenched or masked.

Many treatments have been reported to reduce autofluorescence, including quenching of aldehydes with ammonium sulfate and Tris, bleaching with sodium borohydride, and quenching of autofluorescence with blue or black dyes. The lipophilic dye Sudan Black B is highly effective at masking autofluorescence from lipofuscin, but has the drawback of introducing red and far-red fluorescent background. Our original TrueBlack® Lipofuscin Autofluorescence Quencher (catalog number 23007) was developed as an alternative to Sudan Black B. It effectively quenches lipofuscin autofluorescence with much lower background than Sudan Black B, but still introduces a low level of far-red background. Also, like Sudan Black B, treatment with original TrueBlack® quencher must be performed in 70% ethanol, which is incompatible with some staining protocols and specimen types.

TrueBlack® Plus was developed to allow lipofuscin quenching in aqueous buffer with even lower background than original TrueBlack®. Quenching in PBS allows longer incubation times for thick samples without shrinkage, and is compatible with hydrophobic stains. See Related Products for more background blocking agents and other accessories for immunofluorescence.

Considerations for Use
The following are general considerations for using TrueBlack® Plus Quencher. See Quenching Protocols on the next page for step-by-step instructions for use.

- TrueBlack® Plus is designed to quench lipofuscin, which appears as bright punctate fluorescent granules in tissues like brain and eye from human or aged animals. TrueBlack® Plus may reduce, but not eliminate, autofluorescence from other sources such as red blood cells or extracellular matrix.
- The quencher greatly reduces fluorescence from lipofuscin, but autofluorescence will still be detectable with high imaging gain settings or long exposure times. The concentration of fluorescent probes should be adjusted for optimal signal-to-noise in quencher-treated samples. Tyramide signal amplification (see Related Products) can be used to amplify specific signal over background for low expressing targets and challenging samples such as FFPE human tissue sections.
- When performing immunofluorescence staining of tissue samples, you should include unstained controls with and without quencher to determine the sources of autofluorescence in your sample and the efficacy of quenching. You should also include no primary antibody controls with labeled secondary antibody alone to determine the specificity of antibody staining. Be sure to image the unstained and untreated controls using the same imaging gain setting or exposure time as your stained samples.
- TrueBlack® Plus can be used either before immunofluorescence staining (pre-treatment) or after staining (post-treatment). Post-treatment quenches lipofuscin more effectively, but may also reduce specific fluorescence signal. Pre-treatment does not quench specific signal, but may be less effective for quenching lipofuscin.
- We recommend testing quencher concentrations in the range of 0.5X to 2X to find conditions for optimal signal-to-noise for your experiment. Different incubation times can be tested as well.
- Quenching buffer works best when prepared just before use, however, diluted quencher in PBS can be stored at room temperature for at least 5 days with slight loss of efficacy. Inspect the solution and do not use if precipitation is visible.
- TrueBlack® Plus will be removed from tissues by detergents such as Triton® X-100 or Tween® 20. Care should be taken to wash away all traces of detergent before applying the quencher. If the pre-treatment protocol is used, all buffers used after the quenching step must be free of detergent.
- Buffer rinsing steps should be performed in slide staining jars filled with buffer so that sections are completely submerged.
- TrueBlack® Plus is compatible with aqueous antifade mounting media, including Biotium's EverBrite™, EverBrite™ Hardset, or similar products from other suppliers. TrueBlack® Plus is not compatible with organic-based mounting media like Permount® or DPX mounting medium.
- The protocols provided are intended for researchers with basic knowledge of immunohistochemistry techniques, and describe how to use the quencher on slide-mounted thin paraffin sections or cryosections. The volumes and procedures may be adapted for using the quencher with thick floating sections or other specimens.

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Quenching Protocols

Protocol 1: Post-treatment with TrueBlack® Plus

1. Perform immunofluorescence staining of tissue sections according to your usual protocols. Rinse the sections thoroughly with PBS in a slide staining jar before applying quencher.
2. Vortex the quencher to mix well and centrifuge briefly to collect the solution at the bottom of the vial.
3. Prepare fresh 1X quenching buffer on the day of use by diluting the 40X quencher in PBS at a ratio of 1:40. For example, add 5 uL of 40X quencher to 200 uL of PBS. Vortex to mix well.

   Note: The 40X quencher solution is viscous. Wipe excess solution off the outside of the pipette tip for accurate dispensing. Pipette up and down into the buffer several times to rinse all of the quencher out of the pipette tip and into the buffer.
4. Remove slides from the PBS wash buffer. Tap slides to remove excess buffer and carefully wick away as much buffer as possible from around the sections using a Kimwipe®.
5. Place the slides on a level surface (such as a humidified slide chamber used for antibody incubations) and apply enough 1X quenching buffer to completely cover each section.
6. Incubate for 5-10 minutes at room temperature.
7. Place the slides in a staining jar and rinse three times with PBS.
8. Mount the sections using aqueous-based antifade mounting medium.

Protocol 2: Pre-treatment with TrueBlack® Plus

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 thoroughly with PBS to remove detergent.
3. Prepare fresh 1X quenching buffer on the day of use by diluting the 40X quencher in PBS at a ratio of 1:40. For example, add 5 uL of 40X quencher to 200 uL of PBS. Vortex to mix well.

   Note: The 40X quencher solution is viscous. Wipe excess solution off the outside of the pipette tip for accurate dispensing. Pipette up and down into the buffer several times to rinse all of the quencher out of the pipette tip and into the buffer.
4. Remove slides from the PBS wash buffer. Tap slides to remove excess buffer and carefully wick away as much buffer as possible from around the sections using a Kimwipe®.
5. Place the slides on a level surface (such as a humidified slide chamber used for antibody incubations) and apply enough 1X quenching buffer to completely cover each section.
6. Incubate for 5-10 minutes at room temperature.
7. Place the slides in a staining jar and rinse three times with PBS.
8. Perform immunofluorescence staining according to your usual protocols. 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. Mount the sections using aqueous-based antifade mounting medium.

Related Products

Catalog number	Product
23007	TrueBlack® Lipofuscin Autofluorescence Quencher
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
22020	10X Phosphate-Buffered Saline (PBS)
22030	AntiFix™ Universal Antigen Retrieval Buffer, 10X
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
23012	TrueBlack® IF Background Suppressor System (Permeabilizing)
22010	10X Fish Gelatin Blocking Agent
22011	Fish Gelatin Powder
22014	30% Bovine Serum Albumin Solution
22002	Tween®-20
33000-33020	Tyramide Amplification Kits with CF® Dye or Biotin Tyramide
22029	Tyramide Amplification Buffer Plus
40061	RedDot™2 Far Red Nuclear Counterstain, 200X in DMSO
40043	DAPI in H_2O, 10 mg/mL
40083	NucSpot® 470
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

Please visit our website at www.biotium.com for information on our life science research products, including our full selection of CF® dye and other dye tyramides, 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.

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