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TrueBlack® Lipofuscin Autofluorescence Quencher

#92401


Cell Signaling
TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

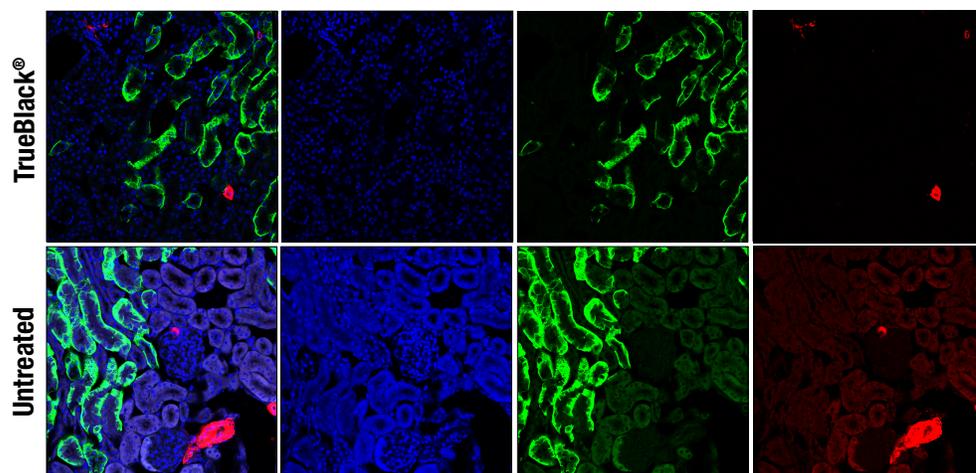
Description: TrueBlack® Lipofuscin Autofluorescence Quencher is specifically formulated to reduce autofluorescence from lipofuscin, a material that can accumulate in aged human and animal tissues. This product can also reduce general background fluorescence, as well as autofluorescence from other sources such as collagen, elastin, and red blood cells.

Specificity/Sensitivity: In addition to significantly reducing autofluorescence, TrueBlack® Lipofuscin Autofluorescence Quencher may cause a decrease in signal with some antibodies by immunofluorescence. We do not find this decrease to be significant enough to limit interpretation of data.

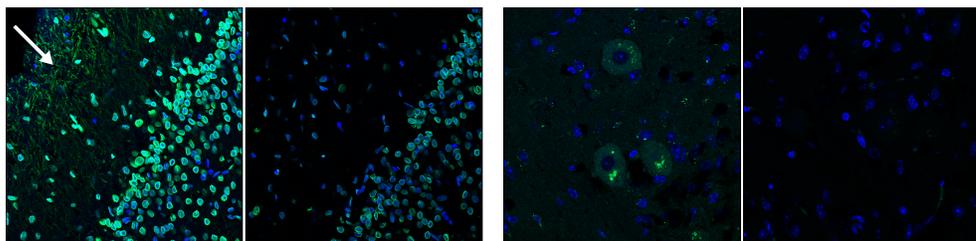
Storage: Store at room temperature. *Protect from light.* This product is stable for 12 months when stored at room temperature.

Safety Information: This product contains Dimethylformamide (DMF). DMF is rapidly absorbed through skin and lungs. It may cause harm to the unborn child. Avoid exposure during pregnancy. If exposed or concerned, get medical advice. See Safety Data Sheet (SDS).

Wear personal protective equipment. Avoid contact with skin. Do not handle until all safety precautions have been read and understood.



Confocal immunofluorescent analysis of fixed frozen mouse kidney, untreated (bottom) or treated with TrueBlack® Lipofuscin Autofluorescence Quencher (top), using AQP4 (D1F8E) XP® Rabbit mAb (Alexa Fluor® 488 Conjugate) #82941 (green), α -Smooth Muscle Actin (D4K9N) XP® Rabbit mAb (Alexa Fluor® 555 Conjugate) #60839 (red), and DAPI #4083 (blue).



Confocal immunofluorescent analysis of fixed frozen mouse midbrain, untreated (left) or treated with TrueBlack® Lipofuscin Autofluorescence Quencher (right), using Lamin A/C (4C11) Mouse mAb (Alexa Fluor® 488 Conjugate) #8617 (green) and DAPI #4083 (blue). White arrow points to autofluorescence.

Confocal immunofluorescent analysis of fixed frozen midbrain from the 5XFAD mouse model of Alzheimer's disease, untreated (left) or treated with TrueBlack® Lipofuscin Autofluorescence Quencher (right), then mounted with ProLong® Gold Antifade Reagent with DAPI #8961 (blue). Green signal shows lipofuscin autofluorescence.

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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry FC-FP—Flow cytometry-Fixed/Permeabilized FC-L—Flow cytometry-Live E-P—ELISA-Peptide
Species Cross-Reactivity: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse
All—all species expected. Species enclosed in parentheses are predicted to react based on 100% homology.

TrueBlack® Lipofuscin Autofluorescence Quencher Protocol

TrueBlack® Lipofuscin Autofluorescence Quencher reacts with lipofuscins to reduce autofluorescence. Consider using this reagent when working with tissue containing lipofuscin (brain, kidney, liver, heart, retina).

IMPORTANT:

- TrueBlack® Lipofuscin Autofluorescence Quencher has been validated with many commonly used counterstains such as DAPI. We recommend testing for compatibility with your reagents of interest prior to full scale experimentation.
- TrueBlack® Lipofuscin Autofluorescence Quencher is not compatible with organic-based mountants.

Required Materials

Supplied

1. **TrueBlack® Lipofuscin Autofluorescence Quencher (#92401):** Supplied as 20X concentrate in DMF.

Not Supplied

1. **70% Ethanol**
2. **1X Wash Buffer (Phosphate Buffered Saline):** To prepare 1L, add 100 mL of 10X Wash Buffer, Phosphate Buffered Saline (#12528) to 900 mL reverse osmosis deionized (RODI) or equivalent grade water. Do not add detergent to this buffer.

Pre-treatment with TrueBlack® Lipofuscin Autofluorescence Quencher

1. When pairing TrueBlack® Lipofuscin Autofluorescence Quencher with an immunostaining experiment, be sure to refer to each antibody's instructions for use. For best results, we recommend processing tissue up until any blocking step and applying TrueBlack® Lipofuscin Autofluorescence Quencher prior to staining.
2. Permeabilize sections as necessary using a buffer compatible with your primary reagent of choice. We recommend a gentle detergent such as Triton™ X-100 diluted to 0.3% in 1X Wash Buffer.

IMPORTANT: TrueBlack® Lipofuscin Autofluorescence Quencher is not compatible with detergent. Any steps involving detergent must be done before applying TrueBlack® Lipofuscin Autofluorescence Quencher.

3. Rinse three times in 1X Wash Buffer for 5 min each to remove detergent.
4. Prepare TrueBlack® Lipofuscin Autofluorescence Quencher solution by diluting 1:20 in 70% ethanol. Vortex to mix.

NOTE: Quenching solution should be made fresh prior to use and discarded if precipitate is visible. We recommend heating the vial of stock solution of TrueBlack® Lipofuscin Autofluorescence Quencher, 20X in DMF to 70°C prior to dilution in order to avoid precipitate formation.

5. Immediately cover tissue sections with 100 µL - 200 µL of quenching solution for 30 seconds at room temperature.

IMPORTANT: Do not allow sections to dry out. Sections may tolerate longer incubations (up to 3 minutes) so long as they remain hydrated.

6. Tap slides on an absorbent towel to collect excess TrueBlack® Lipofuscin Autofluorescence Quencher before transferring to 1X Wash Buffer.
7. Rinse three times in 1X Wash Buffer for 5 min each.
8. Proceed with your staining protocol.