XAF Black™ Ultra Lipofuscin Autofluorescence Blocker

Catalog number: 2468
Unit size: 1 mL

Component	Storage	Amount (Cat No. 2468)
XAF Black™ Ultra Lipofuscin Autofluorescence Blocker	Freeze (< -15 °C), Minimize light exposure	1 mL

OVERVIEW

XAF Black™ Ultra Lipofuscin Autofluorescence Blocker is a recent upgrade of our outstanding XAF Black™ Ultra Lipofuscin Autofluorescence Blocker. It has significantly enhanced quenching capability and greatly improved water solubility. It is specifically formulated to reduce autofluorescence from lipofuscin. XAF Black™ Ultra Lipofuscin Autofluorescence Blocker can be much more effectively used to block the autofluorescence of lipofuscin than other similar products on the market. It can also be used to reduce other background fluorescence, as well as autofluorescence from other sources such as collagen, elastin, and red blood cells. It is a material that can accumulate in aged human and animal tissues. Lipofuscin is a heterogeneous amalgam mainly composed of oxidized proteins (30 to 70%) and lipids such as triglycerides, free fatty acids, cholesterol, and lipoproteins (20 to 50%). Carbohydrates make a small contribution that proportionally may increase with age (4 to 7%). It is generally considered that the protein content has a significant contribution from mitochondria, e.g., ATP synthase subunit residues in congenital ceroid lipofuscinoses. Lipofuscin accumulates in the lysosomes of many cell types with age and/or in patients with severe malnutrition and cancer cachexia. Due to its broad excitation and emission spectra (400 to 700 nm) the presence of lipofuscin complicates the fluorescence imaging of tissues employing exogenous detection fluorophores. The spectrum of lipofuscin overlaps with those of almost all the commonly used detection fluorophores, making it difficult or even impossible to distinguish between specific labelling and autofluorescence caused by lipofuscin. It often hampers fluorescence-based techniques if not properly addressed and corrected for. Autofluorescence is the natural emission of biological substances such as NAD(P)H in liver and vitamin A in hepatic stellate cells. Several other endogenous fluorophores are also known to cause autofluorescence in many tissues.

AT A GLANCE

Protocol Summary

1. Perform fixation, deparaffinization, and antigen retrieval of tissue sections as per your standard protocol.
2. Add XAF Black™ Ultra Lipofuscin Autofluorescence Blocker working solution to the sample.
3. Incubate the sample at room temperature for 5 to 10 minutes.
4. Apply the mounting medium and examine the sample using fluorescence microscopy.

Important

Thaw the XAF Black™ Ultra Lipofuscin Autofluorescence Blocker DMSO solution at room temperature. If any precipitates are observed, gently heat the vial at 37°C for 5 to 10 minutes before preparing the working solution.

PREPARATION OF WORKING SOLUTION

XAF Black™ Ultra Lipofuscin Autofluorescence Blocker Working Solution (1X)

1. To prepare the XAF Black™ Ultra Lipofuscin Autofluorescence Blocker working solution (1X), dilute 25 µL of the XAF Black™ Ultra Lipofuscin Autofluorescence Blocker 40X DMSO stock solution in 1 mL of PBS. Mix thoroughly.

   Note: Protect the working solution from light by covering it with foil or storing it in a dark location

   Note: For optimal results, use this solution within a few hours of preparation.

   Note: Prepare 100 to 200 µL of XAF Black™ Ultra Lipofuscin Autofluorescence Blocker working solution (1X) for each tissue section that will be treated.

SAMPLE EXPERIMENTAL PROTOCOL

Pretreatment with XAF Black™ Ultra Lipofuscin Autofluorescence Blocker

1. Perform fixation, deparaffinization, and/or antigen retrieval of the tissue sections according to your standard protocols.
2. Wash slides with PBS.
3. Remove the PBS, then place the tissues in a humidified slide chamber.
4. Add 100 to 200 µL of XAF Black™ Ultra Lipofuscin Autofluorescence Blocker working solution (1X) to each tissue sample, ensuring the solution fully covers the samples.
5. Incubate the slides at room temperature for 5 to 10 minutes.

   Note: The incubation time may vary based on your specific application and can be adjusted for optimal results.

6. Wash the slides two times with PBS.
7. Perform immunofluorescence staining with antibodies according to your recommended protocol.

   Note: Avoid using buffers containing detergents during blocking, antibody incubation, or washing steps. If detergents are necessary, use them according to the post-treatment protocol.

8. Cover the slides using any aqueous-based fluorescence anti-fade mounting medium, such as FluoroQuest™ PLUS Antifade Mounting Medium (AAT Cat# 20008).

   Note: Avoid using organic-based mounting mediums, as they are incompatible with XAF Black™ Ultra Lipofuscin Autofluorescence Blocker.

Posttreatment with XAF Black™ Ultra Lipofuscin Autofluorescence Blocker

1. Perform fixation, deparaffinization, and/or antigen retrieval of the tissue sections according to your standard protocols.

2. Perform immunofluorescence staining with antibodies according to your recommended protocol.

   Note: Avoid using buffers containing detergents during blocking, antibody incubation, or washing steps. If detergents are necessary, use them according to the post-treatment protocol.

3. Wash the slides with PBS.

4. Remove the PBS, then place the tissues in a humidified slide chamber.

5. Add 100 to 200 µL of XAF Black™ Ultra Lipofuscin Autofluorescence Blocker working solution (1X) to each tissue sample, ensuring the solution fully covers the samples.

6. Incubate the slides at room temperature for 5 to 10 minutes.

   Note: The incubation time may vary based on your specific application and can be adjusted for optimal results.

7. Wash the slides two times with PBS.

8. Cover the slides using any aqueous-based fluorescence anti-fade mounting medium, such as FluoroQuest™ PLUS Antifade Mounting Medium (AAT Cat# 20008).

   Note: Avoid using organic-based mounting mediums, as they are incompatible with XAF Black™ Ultra Lipofuscin Autofluorescence Blocker.

EXAMPLE DATA ANALYSIS AND FIGURES

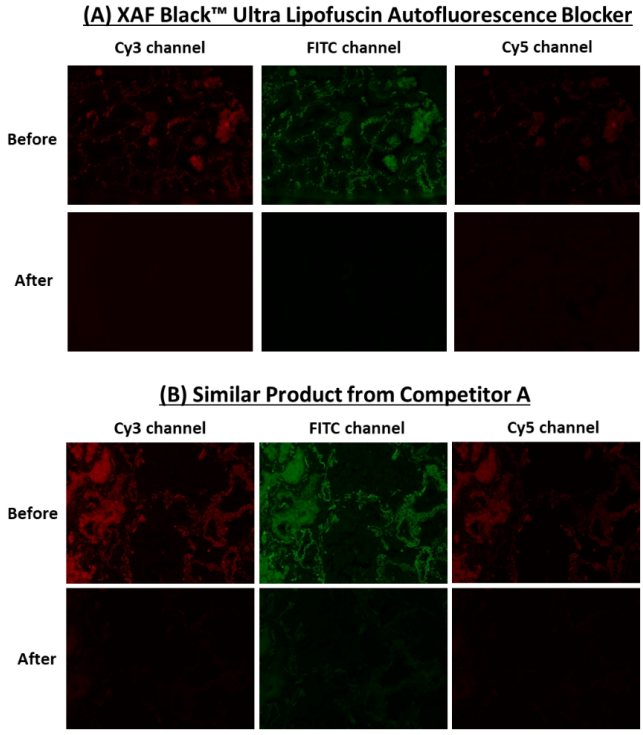

Figure 1. XAF Black™ Ultra Lipofuscin Autofluorescence Blocker effectively diminishes non-lipofuscin autofluorescence in human lung adenocarcinoma tissue sections across the FITC, Cy®3, and Cy®5 fluorescence channels. Imaging was performed using consistent microscope settings across all channels for both control (untreated) and treated tissue sections. Top panel: Pre-treatment; Bottom panel: Post-treatment.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.