XAF Black™ Lipofuscin Autofluorescence Blocker

Catalog number: 2467
Unit size: 1 mL

Component	Storage	Amount (Cat No. 2467)
XAF Black™ Lipofuscin Autofluorescence Blocker	Freeze (< -15 °C)	1 mL

OVERVIEW

XAF Black™ Lipofuscin Autofluorescence Blocker is specifically formulated to reduce autofluorescence from lipofuscin. 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. XAF Black™ Lipofuscin Autofluorescence Blocker can be effectively used to block the autofluorescence of lipofuscin. 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. 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

Important Note

Thaw the XAF Black™ Lipofuscin Autofluorescence Blocker DMSO solution to room temperature. If any precipitates are present, heat the vial at 60–70°C for 5 minutes before preparing the working solution.

Protocol Summary

1. Please follow a standard protocol to perform the following steps: tissue section fixation, deparaffinization, and antigen retrieval.
2. Add the XAF Black™ Lipofuscin Autofluorescence Blocker working solution and incubate at room temperature for 30 to 60 seconds.
3. Add mounting medium to the prepared sample, and then observe it under a fluorescence microscope.

PREPARATION OF WORKING SOLUTION

XAF Black™ Lipofuscin Autofluorescence Blocker Working Solution

1. To prepare the XAF Black™ Lipofuscin Autofluorescence Blocker working solution (1X), mix 50 µL of the XAF Black™ Lipofuscin Autofluorescence Blocker 20X DMSO stock solution with 1 mL of 70% ethanol.

   Note: Protect the working solution from light by either wrapping it in foil or storing it in a dark place.

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

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

SAMPLE EXPERIMENTAL PROTOCOL

Pretreatment with XAF Black™ Lipofuscin Autofluorescence Blocker

1. Follow your standard protocols to perform fixation, deparaffinization, and/or antigen retrieval on tissue sections.
2. Wash slides with PBS.
3. Remove PBS and place the tissue sections in a humidified slide chamber.
4. Add 100 to 200 µL of XAF Black™ Lipofuscin Autofluorescence Blocker working solution (1X) to each tissue sample, ensuring that the entire sample is fully covered.
5. Incubate the slides for 30 to 60 seconds.

   Note: The incubation time can be adjusted based on the requirements of the specific application to achieve optimal results.

6. Wash the slides with PBS twice.
7. Follow your recommended protocol to perform immunofluorescence staining using antibodies.

   Note: It is not recommended to use buffers containing detergents for blocking, antibody incubation, or washing steps. If detergents are necessary, please follow the post-treatment protocol.

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

   Note: It is not recommended to use organic-based mounting mediums, as they are incompatible with the XAF Black™ Lipofuscin Autofluorescence Blocker.

Posttreatment with XAF Black™ Lipofuscin Autofluorescence Blocker

1. Follow your standard protocols to perform fixation, deparaffinization, and/or antigen retrieval on tissue sections.
2. Follow your recommended protocol to perform

immunofluorescence staining using antibodies.

Note: Post-treatment with XAF Black™ Lipofuscin Autofluorescence Blocker may reduce fluorescence signals from antibodies or nuclear stains.

3. Wash slides with PBS.

4. Remove PBS and place the tissue sections in a humidified slide chamber.

5. Add 100 to 200 µL of XAF Black™ Lipofuscin Autofluorescence Blocker working solution (1X) to each tissue sample, ensuring that the entire sample is fully covered.

6. Incubate the slides for 30 to 60 seconds.

   Note: The incubation time can be adjusted based on the requirements of the specific application to achieve optimal results.

7. Wash the slides with PBS twice.

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

   Note: It is not recommended to use organic-based mounting mediums, as they are incompatible with the XAF Black™ Lipofuscin Autofluorescence Blocker.

EXAMPLE DATA ANALYSIS AND FIGURES

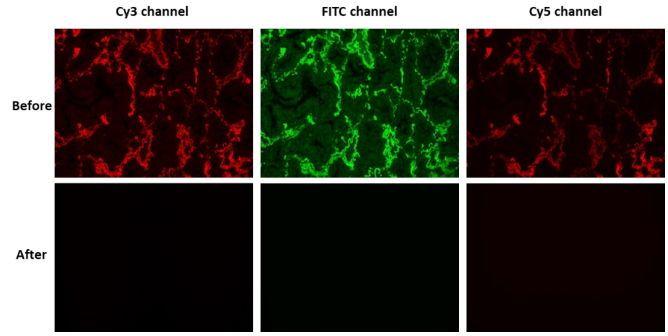

Figure 1. XAF Black™ Lipofuscin Autofluorescence Blocker effectively minimizes non-lipofuscin autofluorescence in human lung adenocarcinoma tissue sections across FITC, Cy®3, and Cy®5 channels. Autofluorescence was imaged using a fluorescence microscope with identical imaging parameters for both untreated and treated samples. The top panel displays tissue sections prior to treatment, while the bottom panel shows the same sections following treatment.

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