Protocol

TD-P Revision 1.0

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Lipofuscin Autofluorescence Quenching Protocol

Using TrueBlack™ Autofluorescence Quencher

Introduction

Lipofuscin consists of autofluorescent granules of oxidized proteins and lipids that build up in the lysosomes of cells as a consequence of aging (Höhn, 2013). 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 reemployed to quench or mask lipofuscin fluorescence.

Traditionally, Sudan Black B has been used to quench lipofuscin autofluorescence by incubating tissue sections with the dye after immunofluorescence staining (Schnell, 1999). 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 (Romijn, 1999). TrueBlack™ is a superior alternative to Sudan Black B for elimination of lipofuscin autofluorescence in tissues such as human brain (Fosso, 2015) and retina (Chan, 2015) with minimal background fluorescence.

TrueBlack™ also reduces autofluorescence from other sources, such as collagen, elastin, red blood cells, and general background fluorescence. 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 of tissue sections can be performed before or after immunostaining. It is rapid, simple, and has minimal effect on signal from fluorescent antibodies or nuclear counterstains.

Storage/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.

Materials

- TrueBlack™ Lipofuscin Autofluorescence Quencher (Catalog # TB-250)
- 70% ethanol (Required, but not supplied)

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Method

Protocol 1 is preferred because it has negligible effect on the signal of fluorescent antibodies and stains. However, buffers containing detergent cannot be used in any the steps after TrueBlack™ treatment, because detergents will remove TrueBlack™ from the tissue. Detergent permeabilization can be performed before TrueBlack™ treatment, but if you need to include detergents during subsequent staining steps, use Protocol 2.

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 20X TrueBlack™ to 1X in 70% ethanol. For example, add 50 µl 20X TrueBlack™ to 1 ml 70% ethanol. Vortex to mix well. Prepare 100-200 µl of 1X TrueBlack™ for each tissue section to be treated.

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™ to completely cover the tissue sections (100-200 µl per section).

   Note: Perform TrueBlack™ treatment on a small number of slides at a time to make sure the sections do not dry out during handling.

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 don't 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 post-treatment protocol.

9. Coverslip the slides using any aqueous-based fluorescence antifade mounting medium.

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Note: TrueBlack™ is not compatible with organic-based mountants like DPX.

Protocol 2: Post-treatment with TrueBlack™

Note: treating with TrueBlack™ after immunostaining may result in lower fluorescence signal from antibodies or nuclear stains.

1. Perform immunostaining according to your standard protocol. Nuclear stains can be added either before or after TrueBlack™ treatment.

2. Prepare 1X TrueBlack™ in 70% ethanol as described in Protocol 1, step 3 above.

3. After the final step of your staining protocol, treat sections with 1X TrueBlack™ as described in Protocol 1, step 4-7 above.

4. After the final step of your staining protocol, treat sections with 1X TrueBlack™ as described in Protocol 1, step 4-7 above.

   Note: TrueBlack™ is not compatible with organic-based mountants like DPX.

References

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