Literature Digest

TrueBlack® Lipofuscin Autofluorescence Quencher in Diverse Neuroscience Applications

The utility of TrueBlack® Lipofuscin Autofluorescence Quencher has been validated in hundreds of peer-reviewed publications. Together, these studies showed that TrueBlack® quencher not only quenched autofluorescence in diverse neuronal and select non-neuronal applications, but often outperformed other quenchers such as Sudan Black B. Along with better signal-to-noise than traditional quenching methods, TrueBlack® quencher also showed less background fluorescence in the far-red spectrum. Here, we compile summaries and takeaway points from three recent publications from unsolicited third parties that illustrate the utility of TrueBlack® quencher in both antibody- and nucleic acid-based assays in diverse neuronal cell types and tissues.

Download our publication list to discover more validated applications for TrueBlack® Lipofuscin Autofluorescence Quencher.

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TrueBlack® Lipofuscin Autofluorescence Quencher Significantly Controls Autofluorescence Inherent to Studies of Microglia

Zhang, H., Tan, C., Shi, X., & Xu, J. (2022). Impacts of autofluorescence on fluorescence-based techniques to study microglia. BMC Neuroscience, 23(1), 1-12. https://doi.org/10.1186/s12868-022-00703-1

Summary

Microglia are a type of neuroglia (glial cell) located throughout the brain and spinal cord that account for 10–15% of all cells found within the brain. As the resident macrophage cells, they play the primary immune role in the central nervous system (CNS). Microglia accrue autofluorescent granules inside their cytoplasm throughout their lifespan which can complicate fluorescence-based analysis.

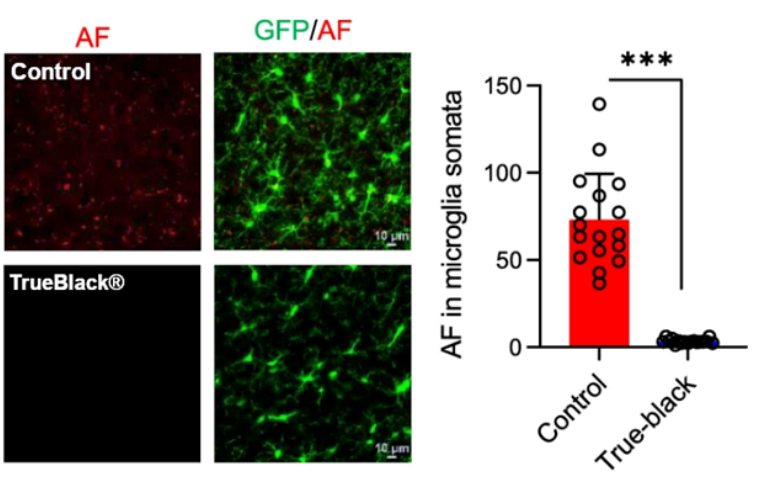
In a recent *BMC Neuroscience* article, Zhang *et al*. report on the impacts of autofluorescence on widely used fluorescence-based techniques to study microglia, including flow cytometry and immunofluorescence staining. An initial failed attempt at using fluorescein isothiocyanate (FITC) conjugated antibodies to detect specific proteins in microglia prompted the team to compare the sensitivities of FITC, phycoerythrin (PE), and allophycocyanin (APC) conjugated antibodies for the detection of surface protein expression in CNS tissues. Using flow cytometry, they found that antibodies conjugated to PE were able to overcome interference from microglia autofluorescence better than FITC and APC conjugated antibodies. The team then used confocal microscopy and identified the source of microglial autofluorescence in fixed brain tissues as cytoplasmic granules that displayed a multi-peak emission spectrum. Due to their widespread presence in CNS tissues, their cellular location, and wide emission spectrum, these autofluorescent granules can be mistaken for genuine antibody signal. Zhang *et al*. tested how to overcome these autofluorescent signals and found that Biotium's TrueBlack® Lipofuscin Autofluorescence Quencher significantly reduced the unwanted signal (Figure 1). In conclusion, the group recommends removing autofluorescence by lipofuscin quenching agents, particularly TrueBlack® quencher, when staining intracellular proteins in microglia with any immunofluorescence-based techniques.

How TrueBlack® lipofuscin quencher was used

TrueBlack® Lipofuscin Autofluorescence Quencher (Cat. No. 23007) was applied to fixed brain slices from 2-month-old *Cx3cr1*^{GFP} mice. Samples were then imaged on a confocal microscope and autofluorescence levels were compared to non-treated control brain slices. TrueBlack® quencher was also used effectively with a panel of antibodies targeting microglial proteins.

Takeaways

- The source of microglial autofluorescence in fixed brain tissues was cytoplasmic granules that displayed a multi-peak emission spectrum.
- Due to their location and wide emission spectrum, these autofluorescent granules can be misidentified as fluorescent signals from immunofluorescence staining.
- TrueBlack® Lipofuscin Autofluorescence Quencher successfully removed microglial autofluorescence.



Imaging of autofluorescence in fixed brain slices. Left: Z-projected image stacks of microglia from *Cx3cr1*^{GFP} mice with autofluorescence imaged in the TRITC channel shown in contrast to slices following treatment with TrueBlack® Lipofuscin Autofluorescence Quencher. Right: Chart showing compiled relative values of autofluorescence from a series of samples like those shown at left (n = 17, from 3 mice, 2 tailed t-test). Bars represent means ± SD. ***P < 0.001. Credit: Zhang, *et al.* https://doi.org/10.1186/s12868-022-00703-1 reproduced under the Creative Commons license.

TrueBlack® Lipofuscin Autofluorescence Quencher Added to Resin Embedding Medium Improves Signal-to-Noise in Brain Tissue

Ren, M., Tian, J., Sun, Q., Chen, S., Luo, T., Jia, X., Jiang, T., Luo, Q., Gong, H., & Li, X., (2021). Plastic embedding for precise imaging of large-scale biological tissues labeled with multiple fluorescent dyes and proteins. Biomed. Opt. Express 12, 6730-6745. https://doi.org/10.1364/BOE.435120

Summary

Studying the anatomy of the brain is a crucial task for understanding how it works. The complex structures work in concert allowing for normal function, and brain diseases can affect the morphology and the relationship of these structures. Given the importance of studying the brain, as well as other whole organs, it is important to develop a standard protocol for observing these organs. Current techniques use resin embedding to preserve structural information of organs in three dimensions. However, the current method of resin embedding is unreliable due to incompatibilities with fluorescent dyes. The existing chemical treatment of resin embedding has led to quenching of fluorescence signal and poor signal-to-noise.

In a recent publication in *Biomedical Optics*, M. Ren *et al*. developed a protocol for improving the existing issues generated by resin embedding of large biological tissues. To reduce background fluorescence, they added TrueBlack® Lipofuscin Autofluorescence Quencher in the glycol methacrylate embedding medium. This method, named glycol methacrylate with TrueBlack® quencher (GMA-T), was used to visualize structures labeled with fluorescent dyes and proteins. Whole brain and brain slices of C57 mice were sectioned using a vibratome either before or after embedding using GMA-T.

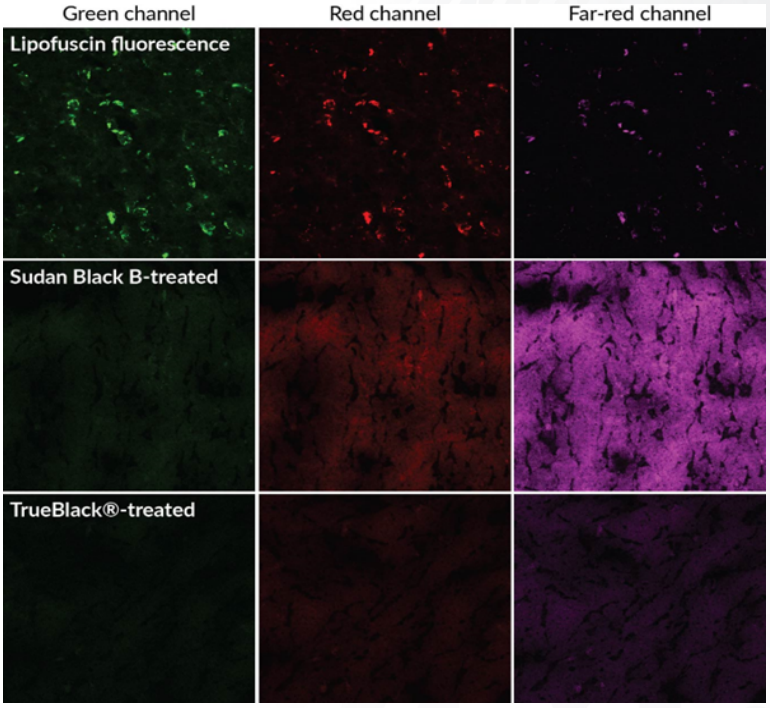
In comparison with the traditionally used autofluorescence quencher, Sudan Black B, TrueBlack® Lipofuscin Autofluorescence Quencher was more successful at inhibiting autofluorescence. The authors found that using 0.7% TrueBlack® quencher in the GMA-T solution successfully inhibited over 90% of background fluorescence, while also retaining much more fluorescent signal than Sudan Black B. They concluded that the use of Sudan Black B was the key factor contributing to poor results with fluorescent dyes in resin embedded samples. However, when Sudan Black B in the resin was replaced with TrueBlack® quencher, fluorescent dyes could be imaged with subcellular resolution. The new approach to resin embedding of organs is important for imaging large-volume biological samples. The implementation of this technique will likely play a useful role in the anatomical study of biological organs going forward.

How TrueBlack® lipofuscin quencher was used

The authors found that using 0.7% TrueBlack® Lipofuscin Autofluorescence Quencher (Cat. No. 23007) in the GMA-T solution successfully inhibited over 90% of background fluorescence while retaining much more fluorescent signal than Sudan Black B.

Takeaways

- Resin embedding of brain tissue can be unreliable due to incompatibilities with fluorescent dyes.
- Ren *et al*. developed a protocol that uses TrueBlack® quencher for improved resin embedding of large biological tissues.
- TrueBlack® quencher was found to be superior to Sudan Black B for reducing background fluorescence in the glycol methacrylate embedding medium.



Lipofuscin autofluorescence in methanol-fixed adult human brain tissue sections. In untreated tissue (top row), lipofuscin appeared as fluorescent granules that fluoresced in all channels. Sudan Black B (middle row) masked lipofuscin, but introduced high background in the red and far-red channels. TrueBlack® quencher (bottom row) masked lipofuscin with minimal increase in background. Credit: Biotium.

TrueBlack® Lipofuscin Autofluorescence Quencher is Preferred for Detection of mRNA in Neuronal Populations

May-Zhang, A. A., Benthal, J. T., & Southard-Smith, E. M. (2022). Hybridization Chain Reaction for mRNA Localization in Single Cells from Mouse and Human Cryosections. Current Protocols, 2(5), e439. https://doi.org/10.1002/cpz1.439

Summary

The incorporation of an isothermal signal amplification mechanism, termed the hybridization chain reaction (HCR), has expanded fluorescence in situ hybridization (FISH) use into more diverse applications. After several iterations, the current third-generation in situ hybridization chain reaction (V3HCR) now allows clear detection of mRNA to subcellular or even single-molecule resolution. However, widespread lipofuscin autofluorescence, most often found in neuronal cell populations, interferes with HCR signal and can complicate analysis.

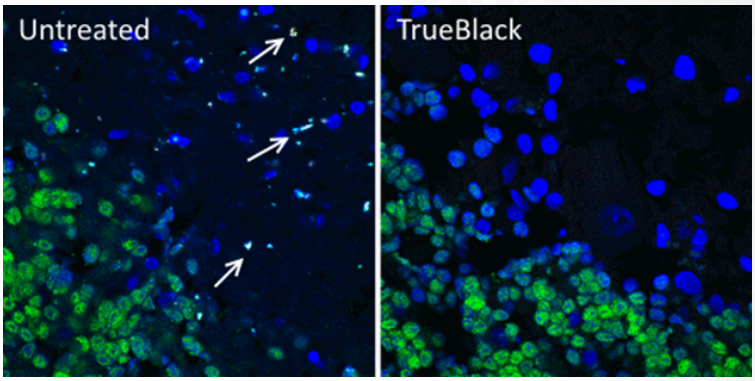
In a recent *Current Protocols* publication, May-Zhang *et al*. report a V3HCR protocol that allows the successful subcellular detection of specific mRNAs in mouse and human tissue cryosections. After cryosectioning and fixing of tissues, the protocol is divided into three discrete day-long segments: 1) hybridization, 2) removal of unbound probe and signal amplification, and 3) washing, counterstaining, and mounting. To demonstrate its efficacy, the protocol was applied to sections of myenteric ganglia in adult mouse and human intestinal tissue to detect marker mRNAs for discrete categories of enteric neurons. After testing a panel of lipofuscin quenching methods, including quenching with cupric sulfate, bleaching in Dent's fixative, detergent extraction, Murray's clearing, treatment with 8% SDS, and standard blocking with Sudan Black B, TrueBlack® Lipofuscin Autofluorescence Quencher was determined to be the only acceptable option for overcoming lipofuscin autofluorescence without causing V3HCR signal loss. Along with demonstrating their detailed protocol in mammalian tissue sections, the authors also beautifully illustrate the difference in detection quality between samples treated with TrueBlack® quencher and non-treated controls. This adds to extensive data showing the critical improvements achieved by using TrueBlack® quencher when preparing samples prone to lipofuscin autofluorescence, such as human neuronal populations.

How TrueBlack® lipofuscin quencher was used

The HCR protocol was applied to sections of myenteric ganglia in adult mouse and human intestinal tissue. A panel of lipofuscin quenching methods were compared, including cupric sulfate, bleaching in Dent's fixative, detergent extraction, Murray's clearing, treatment with 8% SDS, Sudan Black B, and TrueBlack® Lipofuscin Autofluorescence Quencher (Cat. No. 23007).

Takeaways

- The hybridization chain reaction (HCR) has expanded FISH use into more diverse applications.
- Widespread lipofuscin autofluorescence interferes with HCR signal.
- TrueBlack® Lipofuscin Autofluorescence Quencher was found to be the best and only option for overcoming autofluorescence without signal loss.



Quenching of lipofuscin autofluorescence using TrueBlack® Lipofuscin Autofluorescence Quencher pre-treatment before immunofluorescence staining. Formaldehyde-fixed human cortex cryosections were left untreated (left) or treated with TrueBlack® quencher (right), then stained with CF®488A anti-NeuN antibody conjugate (green) and DAPI (blue). Sections were imaged in all channels on a Zeiss LSM700 confocal microscope. Left: Lipofuscin fluoresces brightly in all channels, appearing as white spots (white arrows) in the merged image of untreated tissue. Right: TrueBlack® Lipofuscin Quencher pre-treatment eliminated lipofuscin autofluorescence, with negligible effect on specific staining. Credit: Biotium.

Interested in Learning More About TrueBlack® Background Reducers and Related Products?

Check out these educational pages, references, and other resources available on our website.

Learn More

Learn more about TrueBlack® background reducers

View our other optimized solutions for immunofluorescence microscopy

Learn more about our available primary and secondary antibody conjugates

Tech Tips & Protocols

Tech Tip: Battling Tissue Autofluorescence

Tech Tip: Troubleshooting Tips for Fluorescence Staining

Protocol: Immunofluorescence Staining of Cells for Microscopy

Other Highlighted Citations & References

In situ multiplex immunofluorescence screening for the assessment of tumor immunopathology in pediatric glioblastoma

A new study claims to link oral pathogenic bacteria to Alzheimer's disease pathology

Quenching IF background allows detection of rare disseminated tumor cells (DTCs) in bone marrow

Effective quenching of red blood cell autofluorescence in tissue sections

Click here for a list of TrueBlack® publications

TrueBlack® Lipofuscin Quenchers

Cat. No.	Product name	Unit size
23007	TrueBlack® Lipofuscin Autofluorescence Quencher	1 mL
23014	TrueBlack® Plus Lipofuscin Autofluorescence Quencher	500 uL
23017-23019	EverBrite TrueBlack® Hardset Mounting Medium	10 mL

Other TrueBlack® Background Reducers

Cat. No.	Product name	Unit size
23012	TrueBlack® IF Background Suppressor System (Permeabilizing)	200 assays
22013	TrueBlack® WB Blocking Buffer Kit	50 membranes

Select Immunofluorescence Microscopy Products

Cat. No.	Product name	Unit size
22002	Tween® 20	50 mL
22006	Super^{HT} Pap Pen 4 mm tip, ~800 uses	1 pen
22010	10X Fish Gelatin Blocking Agent	100 mL
22014	30% Bovine Serum Albumin Solution	100 mL
22017	Permeabilization and Blocking Buffer	100 mL
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative	5 x 20 mL
22030	AntiFix™ Universal Antigen Retrieval Buffer, 10X	50 mL
23001	EverBrite™ Mounting Medium	10 mL
23002	EverBrite™ Mounting Medium with DAPI	10 mL
23003	EverBrite™ Hardset Mounting Medium	10 mL
23004	EverBrite™ Hardset Mounting Medium with DAPI	10 mL
23008	Drop-n-Stain EverBrite™ Mounting Medium	10 mL
23009	Drop-n-Stain EverBrite™ Mounting Medium with DAPI	10 mL
40061	RedDot™2 Far Red Nuclear Counterstain, 200X in DMSO	250 uL