

# Fluorescence Background Blockers

TrueBlack® Reagents for IF & Western Blots

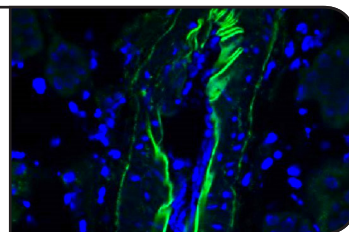
Block autofluorescence and non-specific background for immunofluorescence staining and fluorescent/NIR western blots

Non-specific background fluorescence can have several origins, including sample autofluorescence, non-specific antibody binding, and electrostatic interactions of fluorescent dyes with proteins and blotting membranes. TrueBlack® reagents block unwanted fluorescence from multiple sources, allowing optimal specificity and sensitivity for immunofluorescence staining and western blotting.

## TrueBlack® Products

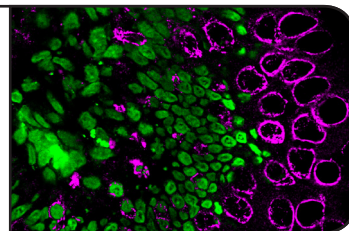
### Lipofuscin Autofluorescence Quencher

- Eliminates lipofuscin autofluorescence
- Reduces background from ECM, blood cells, and other sources
- Stable after mounting in aqueous media



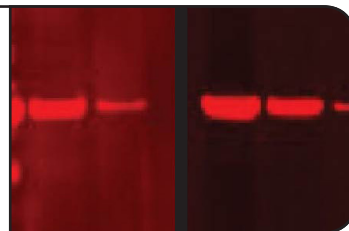
### IF Background Suppressor System

- Suppresses background from multiple sources
- More efficient than Image-iT® FX; block & permeabilize in just 10 minutes
- For cells or tissue sections



### WB Blocking Buffer Kit

- Blocks as well or better than Odyssey® Blocking Buffer, at a lower price
- Excellent choice for phosphoprotein detection
- For visible and near-IR fluorescent western blots



# TrueBlack® Lipofuscin Autofluorescence Quencher

## Lipofuscin can make fluorescence imaging of tissues virtually impossible

Lipofuscin consists of highly autofluorescent granules of oxidized proteins and lipids that build up in the lysosomes of aging cells in a variety of tissues. Lipofuscin granules fluoresce brightly in all channels used for fluorescence microscopy (Figure 1). Consequently, lipofuscin fluorescence must be quenched before specific immunofluorescence staining can be imaged.

## TrueBlack® masks autofluorescence from lipofuscin and other sources, clearing the way for immunofluorescence staining

Traditionally, Sudan Black B dye has been used to mask lipofuscin. However, Sudan Black B also introduces non-specific red and far-red fluorescence, limiting the use of fluorescent dyes in those wavelengths. Biotium developed TrueBlack® as a superior alternative to Sudan Black B to quench autofluorescence with much lower background (Figure 2).

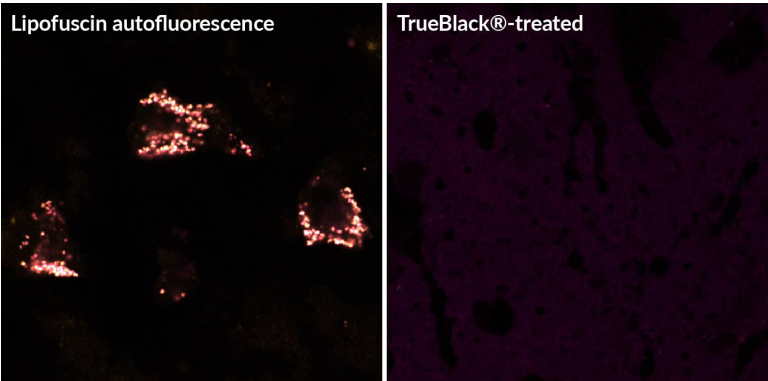


Figure 1. Left: Human brain tissue showing lipofuscin granules with bright, broad-spectrum autofluorescence that appear white in the merged image of the green, red, and far-red channels. Right: Tissue after TrueBlack® treatment, which quenches lipofuscin fluorescence.

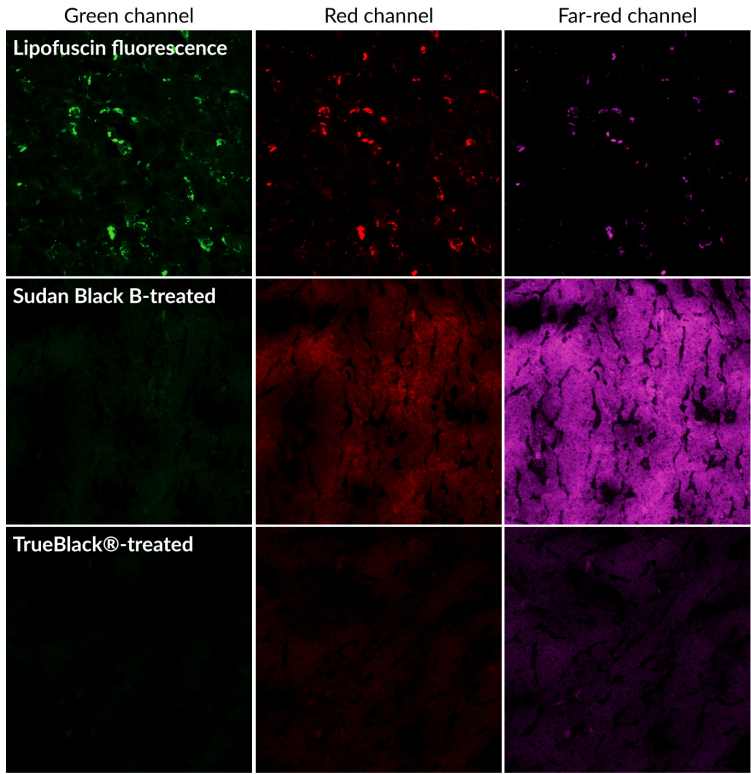


Figure 2. TrueBlack® quenches lipofuscin with less background than Sudan Black B. In untreated human brain tissue (top row), lipofuscin appeared as fluorescent granules in all channels. Treatment with Sudan Black B (middle row) masked lipofuscin, but introduced high background in the red and far-red channels. TrueBlack® treatment (bottom row) eliminated lipofuscin autofluorescence with much lower red/far-red background than Sudan Black B.

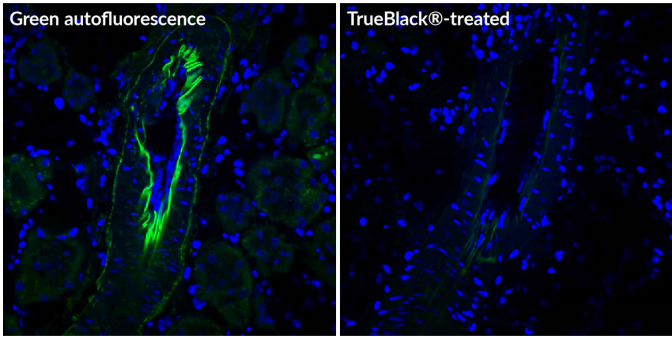


Figure 3. TrueBlack® can reduce autofluorescence from other sources. Rat kidney sections were left untreated or treated with TrueBlack® quencher, then stained with DAPI (nuclei, blue). Untreated tissue showed green autofluorescence from extracellular matrix (left), which was quenched by TrueBlack® (right).

## TrueBlack® reduces autofluorescence from other sources too

TrueBlack® effectively eliminates lipofuscin autofluorescence in tissues like human brain and retina. TrueBlack® also can reduce autofluorescence from other sources, such as blood cells and extracellular matrix (Figure 3). It is not as effective at quenching these sources of autofluorescence as it is for lipofuscin, but it can improve background from multiple sources in human and non-human tissue types (Figure 3). It also has been used to quench fluorescence on polycarbonate filters used as cell supports for imaging.

TrueBlack® treatment can be performed before or after immunostaining (Figure 4). It is rapid, simple, and has minimal effect on signal from fluorescent antibodies or nuclear dyes, thus preserving specific staining. Quenching is stable and compatible with commonly used wet-set and hardset fluorescence mounting media, so slides can be stored after staining.

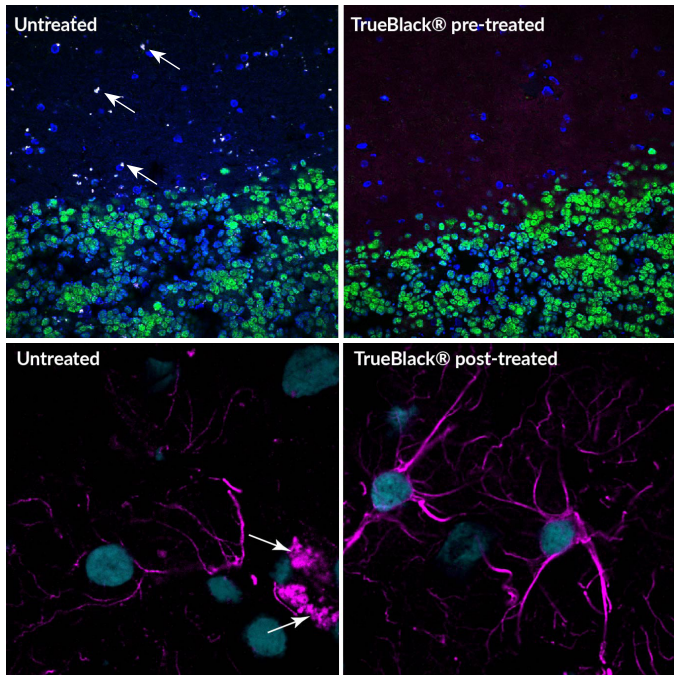


Figure 4. Treatment with TrueBlack® can be performed before or after immunofluorescence staining. Top: TrueBlack® pre-treatment. Human cerebral cortex was left untreated or pretreated with TrueBlack®, then stained with CF®488A anti-NeuN (neuronal nuclei, green) and DAPI (nuclei, blue). In untreated tissue, lipofuscin granules appeared as white speckles (arrows, left) that were quenched by TrueBlack® (right). Bottom: TrueBlack® post-treatment. Human brain sections were stained with rabbit anti-GFAP antibody and CF®640R goat anti-rabbit (glia, magenta) and DAPI (nuclei, blue). The sections were imaged without further treatment (left) or with TrueBlack® treatment (right). Autofluorescent lipofuscin granules in untreated tissue are marked with arrows.



# TrueBlack® IF Background Suppressor System

## Fluorescent dyes can be an unexpected cause of non-specific antibody binding

Non-specific signal in immunofluorescence can arise from multiple sources, including antibody cross-reactivity with off-target proteins, non-specific antibody adsorption to the sample, and tissue autofluorescence. Another cause of background that is not widely known is the effect of fluorescent dyes themselves on the antibody specificity. Next-generation fluorescent dyes like Alexa Fluor® or CF® dyes often carry multiple negative charges to improve dye solubility and brightness of conjugates. However, the extra charge can result in non-specific binding of dye-labeled antibodies. While conventional blocking agents like BSA or gelatin can reduce non-specific protein binding, they don't block background from charged dyes (Figure 5).

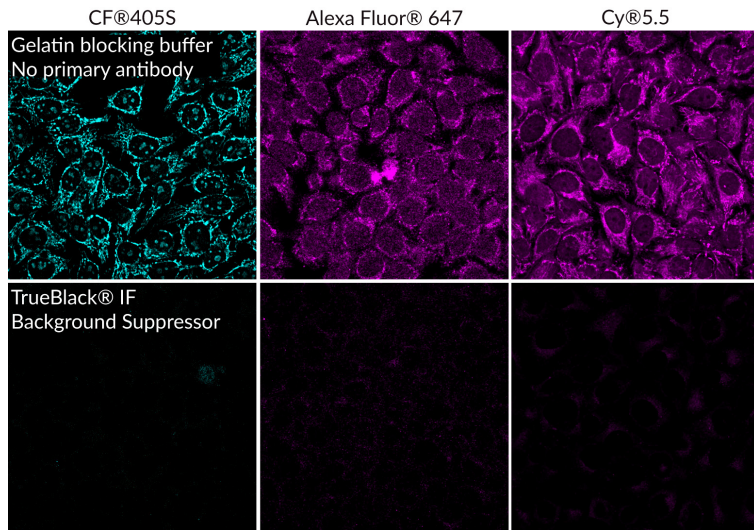


Figure 5. TrueBlack® IF Background Suppressor reduces non-specific binding of antibodies conjugated to charged fluorescent dyes. Methanol-fixed HeLa cells were blocked for 10 minutes with fish gelatin blocking buffer or TrueBlack® IF Background Suppressor, then incubated with goat anti-mouse secondary antibody conjugated to the indicated dyes. Secondary antibody alone gave non-specific background when fish gelatin was used for blocking (top row). Blocking with TrueBlack® IF Background Suppressor resulted in reduced non-specific background (bottom row).

## TrueBlack® Background Suppressor System blocks multiple sources of antibody background

TrueBlack® Background Suppressor includes reagents for blocking both non-specific protein binding as well as background from charged dyes. Examples of charged dyes that show improved signal to noise with the Background Suppressor are CF@405S, CF@405M, CF@555, Alexa Fluor® 647, and Cy@5.5 (Figures 5-6). Background Suppressor also gives excellent results when used with Mix-n-Stain™ labeled primary antibodies (Figures 6-7). One-step blocking and permeabilization takes only 10 minutes, and the buffers contain no mammalian proteins, for broad antibody compatibility.

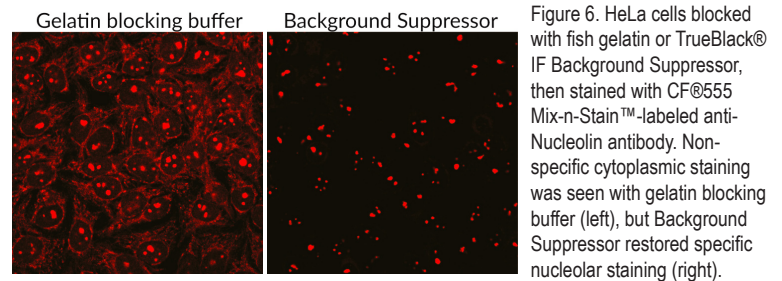


Figure 6. HeLa cells blocked with fish gelatin or TrueBlack® IF Background Suppressor, then stained with CF@555 Mix-n-Stain™-labeled anti-Nucleolin antibody. Non-specific cytoplasmic staining was seen with gelatin blocking buffer (left), but Background Suppressor restored specific nucleolar staining (right).

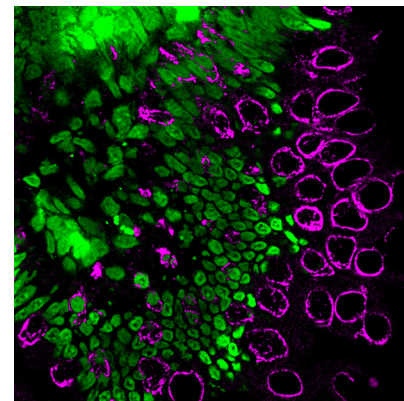


Figure 7. Rat intestine blocked with TrueBlack® IF Background Suppressor and stained with Mix-n-Stain™ CF@488A labeled anti-Histone H1 (green) and Mix-n-Stain™ CF@647 labeled anti-Cytokeratin (magenta).

## TrueBlack® WB Blocking Buffer Kit

### Superior western blocking for next-generation fluorescent dyes

Non-specific signal in WB can arise from multiple sources, including antibody cross-reactivity with off-target proteins, non-specific antibody adsorption to the membrane, and membrane autofluorescence. Another potential cause of background is the effect of fluorescent dyes themselves on the specificity of labeled antibodies. Next-generation fluorescent dyes like Alexa Fluor® or CF® dyes, especially near-infrared dyes, carry negative charge that improves their solubility and brightness. However, the extra charge carried by antibodies labeled with these dyes can result in non-specific binding to proteins and membranes. The TrueBlack® WB Blocking Buffer Kit blocks background from multiple sources including charged dye conjugates (Figure 8). TrueBlack® blocking buffer is especially advantageous for phosphoprotein detection, significantly improving specificity compared to conventional blocking buffers (Figure 9).

TrueBlack® WB Blocking Buffer performs as well or better for fluorescent WB compared to LI-COR® Odyssey® Blocking Buffer (Figure 9), with a lower price per assay.

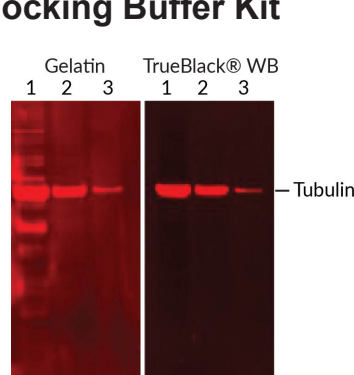


Figure 8. Western blot detection of mouse anti-tubulin with Alexa Fluor® 790 goat anti-mouse. Membranes were blocked with fish gelatin or TrueBlack® WB Blocking Buffer. The highly negatively charged Alexa Fluor® 790 conjugate showed non-specific binding to PVDF and proteins, which was blocked by TrueBlack® WB Blocking Buffer. Lanes 1-3: 10 ug, 1 ug, or 0.1 ug HeLa cell total protein. Note: Alexa Fluor® 790 was used to demonstrate non-specific background from highly charged dyes. Biotium's near-IR CF® dyes have structural features that result in significantly lower background.

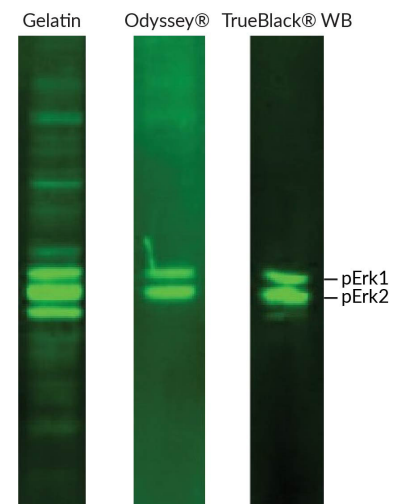


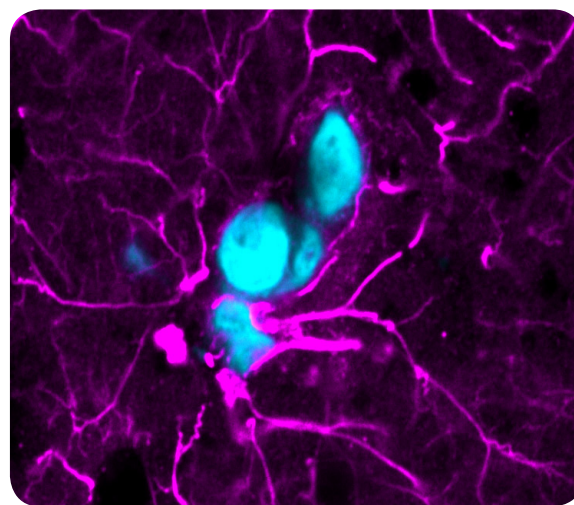
Figure 9. Western blot detection of phospho-Erk1/2 in PDGF-stimulated NIH-3T3 cell lysate. Membranes were blocked with fish gelatin, LI-COR® Odyssey® TBS Blocking Buffer, or TrueBlack® WB Blocking Buffer. Rabbit anti-pErk1/2 and CF@680R donkey anti-rabbit antibodies were used for detection. TrueBlack® WB Blocking Buffer gave lower background fluorescence and better specificity.

# TrueBlack® Background Reducers

For immunofluorescence and fluorescent/NIR western

## Ordering Information

| Cat. No. | Size             | Product   |
|----------|------------------|---|
| 23007    | 1 mL             | TrueBlack® Lipofuscin Autofluorescence Quencher, 20X in DMF |
| 23012-T  | 20 assays        | TrueBlack® IF Background Suppressor System (Permeabilizing) |
| 23012    | 200 assays       |   |
| 23013-T  | For 10 membranes | TrueBlack® WB Blocking Buffer Kit                           |
| 23013    | For 50 membranes |   |



## More immunofluorescence essentials from Biotium

| Cat. No. | Product Name                                       | Description   |
|----------|--|---|
| 40061    | RedDot™2 Far Red Nuclear Counterstain              | Far-red nuclear stain for fixed cells or selective dead cell staining   |
| 40083    | NucSpot® 470 Nuclear Stain                         | Green fluorescent nuclear stain for fixed cells or selective dead cell staining   |
| 23001    | EverBrite™ Mounting Medium                         | Superior wet-set antifade medium compatible with wide range of fluorescent dyes, with or without DAPI for nuclear counterstaining         |
| 23002    | EverBrite™ Mounting Medium with DAPI               |   |
| 23003    | EverBrite™ Hardset Mounting Medium                 | Forms a stable hard seal with excellent protection against photobleaching for fluorescent dyes, with or without DAPI nuclear counterstain |
| 23004    | EverBrite™ Hardset Mounting Medium with DAPI       |   |
| 23005    | CoverGrip™ Coverslip Sealant                       | Only product specifically designed for sealing the edges of wet-mounted coverslips  |
| 22005    | Mini Super <sup>HT</sup> Pap Pen 2.5 mm tip        | Create hydrophobic barriers on slides to conserve antibody; stable to 120°C, available in two sizes                                       |
| 22006    | Super <sup>HT</sup> Pap Pen 4 mm tip               |   |
| 22023    | Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative | High purity, methanol-free, ready-to-use formaldehyde fixative  |
| 22016    | Permeabilization Buffer                            | Detergent-based permeabilization buffer for intracellular immunofluorescence staining   |
| 22017    | Permeabilization and Blocking Buffer               | Goat serum-based blocking and permeabilization buffer   |
| 22010    | 10X Fish Gelatin Blocking Agent                    | Fish gelatin solution to add to your preferred buffer for IF or western blot blocking   |
| 22014    | Bovine Serum Albumin 30% Solution in Water         | For use as a blocking agent or protein stabilizer; prepared from IgG- and protease-free BSA   |
| 22020    | 10X Phosphate-Buffered Saline (PBS)                | Molecular biology grade (RNase-, DNase-, protease-free), supplied in 4L Cubitainer®   |
| 41024-4L | Water, Ultrapure Molecular Biology Grade           | Ultrafiltered to eliminate RNase, DNase, protease, pyrogens/endotoxins and bacteria; non-sterile  |
| 92400    | Mix-n-Stain™ CF®680T Total Protein Prestain Kit    | Highly linear protein quantitation for accurate western normalization, available in two near-infrared colors for 700 or 800 nm detection  |
| 92401    | Mix-n-Stain™ CF®770T Total Protein Prestain Kit    |   |
| 21530    | Peacock™ Prestained Protein Marker                 | Ready-to-use protein markers with eight or ten bands in three colors for easy identification on SDS-PAGE gels or western blots            |
| 21531    | Peacock™ Plus Prestained Protein Marker            |   |

Biotium carries a full line of fluorescent probes, including next generation CF® dye labeled primary antibodies, secondary antibodies, phalloidins, antibody labeling kits, and many other reagents for live cell imaging, cell viability, and molecular biology.

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