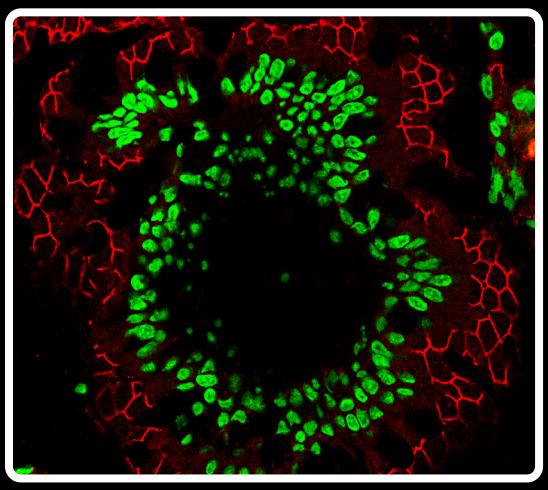


Fluorescence Background Blockers

TrueBlack® Reagents for IF & Western Blots

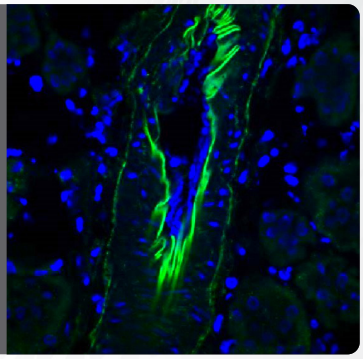
Block lipofuscin autofluorescence and non-specific background fluorescence for immunofluorescence staining and fluorescent/near-IR western blots

Non-specific background fluorescence can have several origins, including lipofuscin, 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.

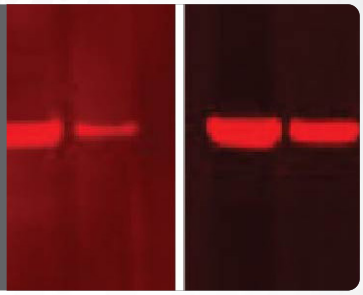
Lipofuscin Autofluorescence Quenchers

- Effectively quenches lipofuscin autofluorescence
- Reduces background from ECM, blood cells, and other sources
- Stable after mounting in aqueous media
- TrueBlack® Plus is the only quencher that works in aqueous buffer



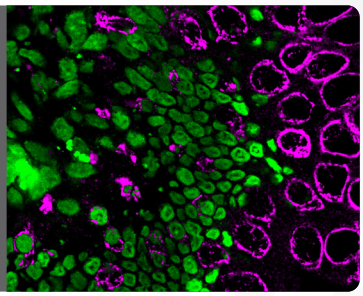
WB Blocking Buffer Kit

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



IF Background Suppressor System

- Suppresses background from non-specific antibody binding and charged fluorescent dyes
- More efficient than Image-iT® FX; block & permeabilize in just 10 minutes
- For cells and tissue sections



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TrueBlack® Lipofuscin Autofluorescence Quenchers

TrueBlack® quenchers effectively eliminate autofluorescence from lipofuscin in tissues

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 (Fig. 1). Lipofuscin granules fluoresce brightly in all channels used for fluorescence microscopy. Consequently, lipofuscin fluorescence must be quenched before imaging cellular structures or specific immunofluorescence targets.

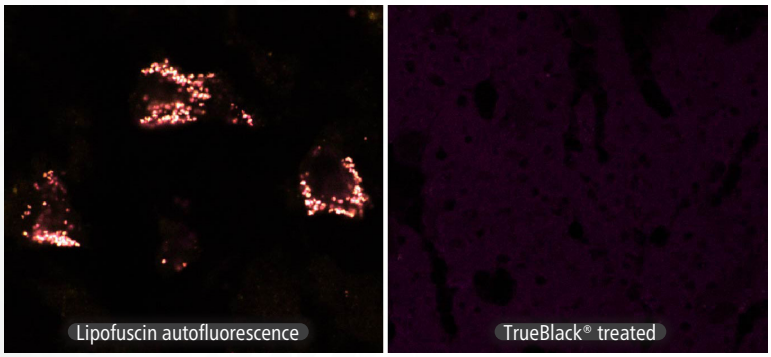


Figure 1. Left: Human brain tissue showing lipofuscin granules with bright, broad-spectrum autofluorescence that appear white in the merged image of the green, red, and far-red channels. Right: Tissue after original TrueBlack® treatment, which quenches lipofuscin autofluorescence.

NEW! TrueBlack® Plus is the only lipofuscin quencher that can be used in aqueous buffers

TrueBlack® Plus is a next-generation lipofuscin quencher developed by Biotium chemists to allow lipofuscin quenching in aqueous buffer with even lower background than the original TrueBlack® (Fig. 2). Quenching in PBS allows longer incubation times for thick samples without shrinkage, and is compatible with hydrophobic stains.

TrueBlack® and TrueBlack® Plus Features

- Quench lipofuscin autofluorescence and reduce background from ECM, blood cells, and other sources
- Lower background than Sudan Black B
- Compatible with immunofluorescence, immunohistochemistry, and in situ hybridization workflows
- Use before or after immunostaining
- Stable after mounting in most commonly used aqueous mounting media
- Minimal effect on signal from fluorescent antibodies or nuclear stains

TrueBlack® and TrueBlack® Plus quench lipofuscin with lower background than Sudan Black B

Traditionally, Sudan Black B has been used to quench lipofuscin autofluorescence by incubating tissue sections with the dye after immunofluorescence staining. However, Sudan Black B also introduces non-specific red and far-red fluorescence, limiting the use of fluorescent dyes in those wavelengths (Fig. 2). Biotium's TrueBlack® and TrueBlack® Plus are superior alternatives to Sudan Black B to quench autofluorescence with much lower background in all channels.

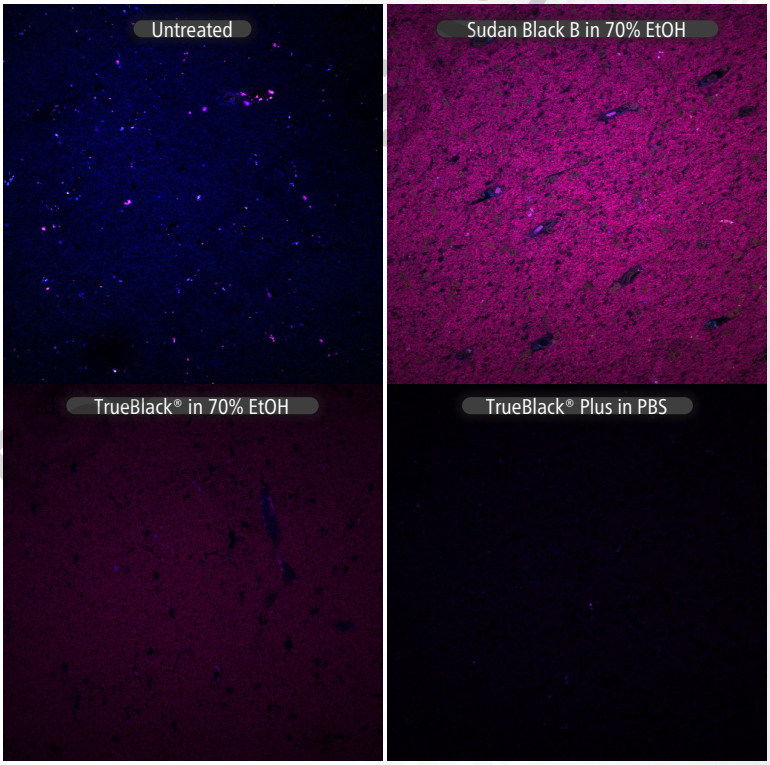


Figure 2. Lipofuscin autofluorescence in methanol-fixed human cerebral cortex cryosection without quenching treatment appears as bright punctate spots that fluoresce in all channels (top left), appearing pink in the merged image. Traditional treatment with Sudan Black B introduces high levels of far-red background fluorescence (top right). Treatment with original TrueBlack® eliminates lipofuscin autofluorescence, but still introduces low-level red and far-red fluorescence (bottom left). TrueBlack® Plus can be used in PBS instead of 70% ethanol, and quenches lipofuscin autofluorescence with the lowest level of red and far-red background (bottom right).

TrueBlack® and TrueBlack® Plus reduce autofluorescence from other sources too

TrueBlack® and TrueBlack® Plus can also reduce autofluorescence from other sources, such as red blood cells and extracellular matrix. It is not as effective at quenching these sources of autofluorescence as it is for lipofuscin, but it can improve background in human and non-human tissue types.

TrueBlack® treatment can be performed before or after immunostaining. 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. TrueBlack® has also been used to quench autofluorescence on polycarbonate filters used as cell supports for imaging.

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TrueBlack® or TrueBlack® Plus?

Choose the right autofluorescence quencher for your sample and workflow

Product	Pros	Cons
TrueBlack® Lipofuscin Autofluorescence Quencher	• Complete quenching of lipofuscin autofluorescence • Lower red & far-red background than Sudan Black B • Ultra-low background in blue and green channels • Quenching takes only 30 seconds • Widely published	• Introduces some red & far-red background • Quenching must be done in 70% EtOH • Some quenching of fluorescent dyes • Not compatible with organic solvent-based mounting media
TrueBlack® Plus Lipofuscin Autofluorescence Quencher	• Greatly reduces lipofuscin autofluorescence • Lower red & far-red background than Sudan Black B and original TrueBlack® • The only lipofuscin quencher that can be used in aqueous buffers like PBS	• Titration recommended for optimal quenching • May not be as effective as the original TrueBlack® for samples with high lipofuscin • Some quenching of fluorescent dyes • Not compatible with organic solvent-based mounting media

TrueBlack® IF Background Suppressor System

A buffer system designed for optimal blocking of non-specific staining in immunofluorescence

Non-specific signal in immunofluorescence can arise from multiple sources: 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.

Fluorescent dyes like Alexa Fluor® or CF® dyes often carry multiple negative charges to improve dye solubility and brightness of conjugates. However, the 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 are not effective at blocking background from charged dyes (Fig. 3).

IF Background Suppressor System Features

- Suppresses background from non-specific antibody binding and charged fluorescent dyes
- More efficient than Image-iT® FX; block and permeabilize in just 10 minutes
- For cells or tissue sections
- Combine with TrueBlack® or TrueBlack® Plus to reduce non-specific signal and sample background fluorescence

TrueBlack® IF 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. Background Suppressor gives excellent results when used with dye labeled primary antibodies.

One-step blocking and permeabilization takes only 10 minutes; and the buffers contain no mammalian proteins for broad antibody compatibility.

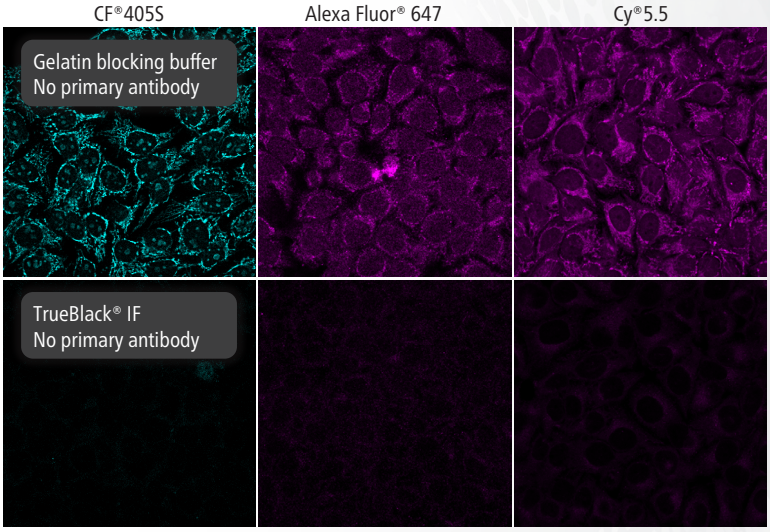


Figure 3. TrueBlack® IF Background Suppressor System reduces non-specific binding of antibodies conjugated to charged fluorescent dyes. Methanol-fixed HeLa cells were blocked for 10 minutes with fish gelatin blocking buffer or TrueBlack® IF Background Suppressor, then incubated with goat anti-mouse secondary 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 System resulted in reduced non-specific background (bottom row).

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TrueBlack® WB Blocking Buffer Kit

Superior western blocking for fluorescent dyes

Non-specific signal in western blotting (WB) can arise from multiple sources: 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. Fluorescent dyes like Alexa Fluor® or CF® dyes, especially near-infrared dyes, carry negative charge that improves their solubility and brightness. However, the charge carried by antibodies labeled with these dyes can result in non-specific binding to proteins and membranes.

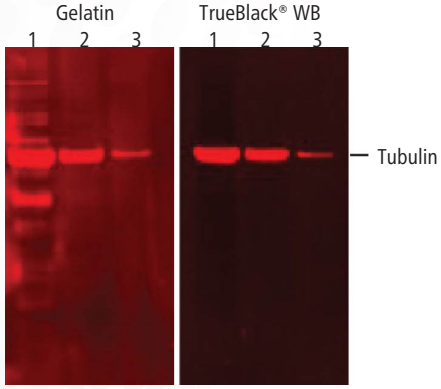


Figure 4. Western blot detection of mouse anti-tubulin with Alexa Fluor® 790 goat anti-mouse. Membranes were blocked with fish gelatin or TrueBlack® WB Blocking Buffer. The negatively charged Alexa Fluor® 790 conjugate showed non-specific binding to PVDF and proteins, which was blocked by TrueBlack® WB Blocking Buffer. Lanes 1-3: 10 ug, 1 ug, or 0.1 ug HeLa cell total protein.

WB Blocking Buffer Kit Features

- 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® WB Blocking Buffer performs better than other blocking buffers and reagents

The TrueBlack® WB Blocking Buffer Kit blocks background from multiple sources including charged dye conjugates. TrueBlack® WB Blocking Buffer significantly improves antibody specificity compared to gelatin in a western blot to detect tubulin using an antibody labeled with Alexa Fluor® 790 (Fig. 4). 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. Moreover, TrueBlack® WB Blocking Buffer significantly improves specificity for phosphoprotein detection compared to conventional blocking buffers (Fig. 5).

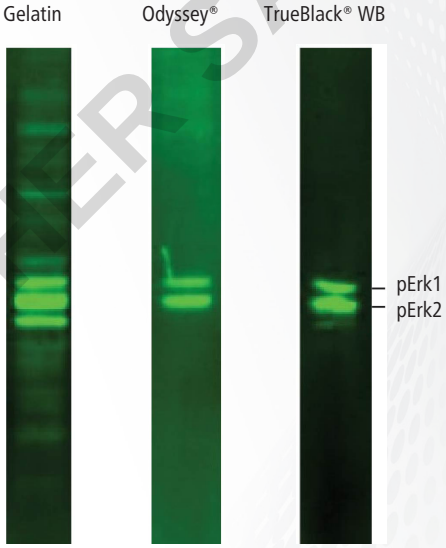


Figure 5. Western blot detection of phospho-Erk1/2 in PDGF-stimulated NIH-3T3 cell lysate. Membranes were blocked with fish gelatin, LI-COR® Odyssey® TBS Blocking Buffer, or TrueBlack® WB Blocking Buffer. Rabbit anti-pErk1/2 and CF®680R donkey anti-rabbit antibodies were used for detection. TrueBlack® WB Blocking Buffer gave lower background fluorescence and better specificity.

Ordering Information

Cat. #	Size	Product	Applications
23007	1 mL	TrueBlack® Lipofuscin Autofluorescence Quencher, 20X in DMF	Quench lipofuscin autofluorescence, improve non-specific fluorescent background in tissue, compatible with immunofluorescence, in situ hybridization, and immunohistochemistry workflows.
23014-T	50 uL	TrueBlack® Plus Lipofuscin Autofluorescence Quencher, 40X in DMSO	
23014	500 uL		
23012-T	20 Assays	TrueBlack® IF Background Suppressor System (Permeabilizing)	Reduce background from non-specific antibody binding and charged fluorescent dyes in immunofluorescence, in situ hybridization, and immunohistochemistry
23012	200 Assays		
23013-T	For 10 membranes	TrueBlack® WB Blocking Buffer Kit	Reduce background from non-specific antibody binding and charged fluorescent dyes in visible and near-IR fluorescent western blotting
23013	For 50 membranes		

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. Visit biotium.com for information about our high quality fluorescent reagents.

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