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- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Product Information

TrueBlack® WB Blocking Buffer Kit

Component	23013-T 10 membranes*	23013 50 membranes*
23013A: TrueBlack® WB Blocking Buffer	100 mL	500 mL
23013B: TrueBlack® WB Antibody Diluent	200 mL	1 L

*Number of membranes based on 10 mL per incubation; actual number of membranes may vary depending on protocol used and membrane size.

Storage and Handling

Store at 4°C. Product is stable for at least 12 months from date of receipt when stored as recommended.

Appearance

23013A TrueBlack® WB Blocking Buffer: Colorless solution. It is normal for the Blocking Buffer to be slightly turbid at room temperature, which does not affect its performance. Storing at 4°C may cause increased turbidity and precipitation. Warm up at room temperature and shake to mix before using.

23013B TrueBlack® WB Antibody Diluent: Colorless solution.

Product Description

The TrueBlack® WB Blocking Buffer Kit is a ready-to-use buffer system for fluorescence-based western blot (WB). The buffers are designed to achieve optimal specificity and sensitivity by blocking background from the non-specific interaction between the fluorophore-labeled antibodies and the membrane with cell lysates.

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 that is not well-known is the effect of fluorescent dyes themselves on the specificity of labeled antibodies. 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 carried by the dye can result in non-specific antibody binding and background fluorescence. While conventional blocking agents like BSA, gelatin, or casein can reduce non-specific protein binding, they are not effective at blocking background from charged dyes.

The TrueBlack® WB Blocking Buffer Kit is designed to block both non-specific protein binding as well as background from charged dyes. Compared to a regular gelatin-based blocking buffer, the TrueBlack® WB Blocking Buffer Kit not only suppresses background fluorescence over the entire membrane, but also eliminates non-specific bands (see Figure 1A). The TrueBlack® WB Blocking Buffer Kit is especially advantageous for phosphoprotein detection, significantly improving specificity compared to the gelatin-based blocking buffer (see Figure 1B).

The buffers in the TrueBlack® WB Blocking Buffer Kit contain non-mammalian based blocking agents. The kit can be used with antibodies labeled with all types of fluorophores spanning the visible and near-infrared (NIR) spectrum. The kit is compatible with both polyvinylidene difluoride (PVDF) and nitrocellulose membranes. The kit is NOT designed for chemiluminescence detection using horseradish peroxidase (HRP) conjugated antibodies.

The TrueBlack® WB Blocking Buffer Kit belongs to our TrueBlack® line of background reducing agents for fluorescence applications, which includes TrueBlack® Lipofuscin Autofluorescence Quencher and TrueBlack® IF Background Suppressor System (Permeabilizing) (see Related Products).

General Considerations for Fluorescent Western Detection

- Fluorescence western detection requires an imaging system capable of detecting fluorescent dyes in specific channels. For best results, use a gel imager or scanner specifically designed for imaging fluorescent blots.
- NIR fluorescent dyes such as CF®680 and CF®770 Dyes are optimal for fluorescent western, because background is lower in these wavelengths. Visible fluorescent dyes can be used, but generally will have lower signal-to-noise ratios due to higher autofluorescence of proteins and blotting membranes in the visible spectrum.
- For protein markers we recommend using Peacock™ Prestained Protein Markers which fluoresce in the 700 channel and range from 8 kDa to 245 kDa (see Related Products).
- Blue tracking dyes in SDS-PAGE loading buffer can fluoresce in the far-red/NIR spectra; loading buffer with an orange tracking dye is recommended for fluorescent western detection.
- We recommend using low-fluorescence PVDF for fluorescent western blot detection. Nitrocellulose membranes may also be used and in our experience have shown similar background fluorescence to low-fluorescence PVDF.
- Ponceau S Solution is not recommended for fluorescent western blots due to its poor sensitivity for low loading amounts, especially on PVDF. For total protein staining, we recommend VersaBlot™ Total Protein Normalization Kits due to their exceptional linearity, ease-of-use, and downstream reversibility for multi-color analysis (see Related Products).
- 9 cm^2 petri dishes hold 5-10 mL and are convenient for washing and incubating mini-blots. Alternatively, commercially available black blotting boxes for fluorescent westerns come in a variety of sizes for blots or membrane strips.
- Either the TrueBlack® WB Blocking Buffer or the TrueBlack® WB Antibody Diluent may be used to dilute antibodies for staining. We have found that diluting antibodies in either solution works well. However, we recommend testing each buffer to find the optimal conditions for your antibody. Please note, choosing to dilute your antibodies in fresh TrueBlack® WB Blocking Buffer will cut the number of assays supplied by the kit in half.
- Either phosphate-buffered saline (PBS) or Tris-buffered saline (TBS) can be used for fluorescent western detection with similar results.
- It may be desirable to minimize the volume of antibody solutions used for blotting by using sealable bags or small containers. Enough solution should be used to freely move across the blot without trapping bubbles.
- For blocking and wash steps, don't skimp on volume. Use 5-10 mL buffer for a mini-blot. The blot should move freely in the buffer.

Table 1. Comparison between the TrueBlack® WB Blocking Buffer Kit and the Odyssey® Blocking Buffer

Product	TrueBlack® WB Blocking Buffer Kit	Odyssey® Blocking Buffer*
Trial Size	For 10 membranes	125 mL for 4 membranes
Full Size	For 50 membranes	500 mL for 16 membranes

*According to the 'Odyssey® Western Blot Blocker Optimization for Near-Infrared (NIR) Detection' by LI-COR Inc, it is recommended to use the Odyssey® Blocking Buffer for blocking and antibody dilution.

Experimental Protocols

Here we provide a reference protocol for primary and secondary antibody binding. You may need to adjust the time and temperature of incubation and washing for your specific application.

Materials required but not provided

Either TBS-T or PBS-T may be used with fluorescent western blots.

- TBS-T (25 mM Tris, 150 mM NaCl, 0.05% TWEEN® 20 in water, pH = 7.5)
- PBS-T (1X phosphate-buffered saline, 0.05% TWEEN® 20, pH = 7.5)

Fluorescent western blotting procedure

1. Run protein gel and transfer to nitrocellulose or PVDF membrane following standard WB procedures.
2. Warm up the TrueBlack® WB Blocking Buffer to room temperature and shake to thoroughly mix before using.

   Note: It is normal for the TrueBlack® WB Blocking Buffer to be slightly turbid with a small amount of white precipitate, this does not affect performance.
3. After transfer, incubate the membrane in TrueBlack® WB Blocking Buffer at room temperature for 45 minutes. Use ~ 10 mL of buffer to cover a 7 x 8.5 cm membrane for mini-gels.
4. Remove the Blocking Buffer and add primary antibodies diluted with TrueBlack® WB Antibody Diluent to the membrane. Alternatively, you can also add primary antibody directly to the Blocking Buffer on the membrane and mix well. Multiple primary antibodies from different species can be diluted together. Consult the manufacturer of the primary antibodies for optimal dilution ratio.
5. Incubate at room temperature for 2 hours or 4°C overnight. We recommend 4°C overnight for optimal sensitivity and specificity.
6. Remove the primary antibodies and wash the membrane 5 x 10 minutes with TBS-T.
7. Add fluorescent dye-labeled secondary antibodies diluted with TrueBlack® WB Antibody Diluent to the membrane. Multiple secondary antibodies can be diluted together. Consult the manufacturer of the secondary antibodies for optimal dilution ratio.

   Note: We recommend diluting CF® Dye secondary antibodies (2 mg/mL) 1:20,000 in TrueBlack® WB Antibody Diluent, for a final concentration of 0.1 ug/mL.
8. Incubate at room temperature for 2 hours, protected from light.
9. Remove the secondary antibodies and wash the membrane 5 x 10 minutes with TBS-T, protect from light.
10. If a tertiary antibody, streptavidin, or lectin binding is needed, follow steps 7 - 9.
11. The membrane is ready for detection using a fluorescence imager.

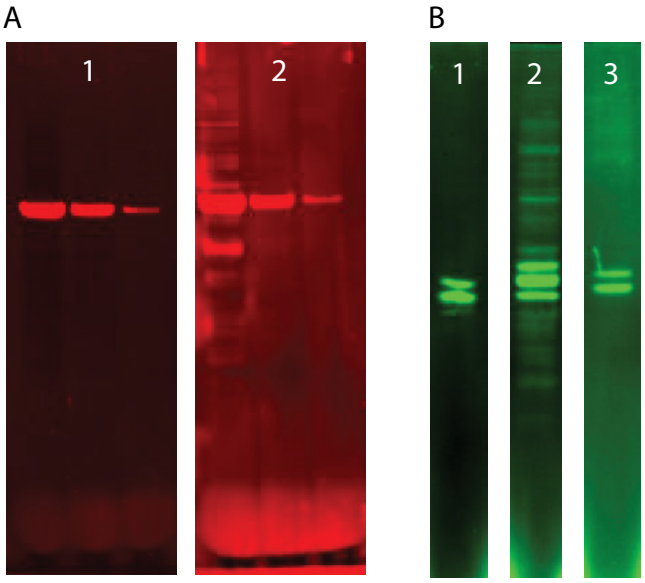

Figure 1. WB results using the TrueBlack® WB Blocking Buffer Kit (membrane 1) compared to a gelatin-based blocking buffer (membrane 2) and the Odyssey® Blocking Buffer (membrane 3). (A) Tubulin was detected using mouse anti-tubulin primary antibody and Alexa Fluor® 790 goat anti-mouse secondary antibody. On each membrane, 10, 1, and 0.1 ug of HeLa cell lysate was loaded in each lane, from left to right. (B) Phosphorylated extracellular signal-regulated kinases (pERK, showing two isoforms) were detected using rabbit anti-pERK primary antibody and CF®680R donkey anti-rabbit secondary antibody. In both (A) and (B), membrane 1 was developed using the TrueBlack® WB Blocking Buffer Kit; membrane 2 was developed using 2% fish gelatin in TBS-T as blocking reagent and antibody diluent. Membrane 3 in (B) was developed using the Odyssey® Blocking Buffer (TBS) by LI-COR Inc. Membrane 1 shows lower background fluorescence as well as fewer non-specific bands compared to membrane 2 and 3.

Related Products

Catalog number	Product
23007	TrueBlack® Lipofuscin Autofluorescence Quencher
23014	TrueBlack® Plus Lipofuscin Autofluorescence Quencher
23012	TrueBlack® IF Background Suppressor System (Permeabilizing)
33025, 33026	VersaBlot™ Total Protein Normalization Kits
21003	One-Step Blue® Protein Gel Stain
21004	One-Step Lumitein™ Protein Gel Stain
21005	One-Step Lumitein™ UV Protein Gel Stain
21530	Peacock™ Prestained Protein Marker
21531	Peacock™ Plus Prestained Protein Marker
20192	CF®680R Goat Anti-Mouse IgG (H+L), highly cross-adsorbed
20193	CF®680R Goat Anti-Rabbit IgG (H+L), highly cross-adsorbed
20078	CF®770 Goat Anti-Rabbit IgG (H+L), highly cross-adsorbed
20077	CF®770 Goat Anti-Mouse IgG (H+L), highly cross-adsorbed
29072	CF®680R Streptavidin Conjugate
22002	Tween®-20
90082	DMSO, Anhydrous
22014	Bovine Serum Albumin 30% Solution
22010	10X Fish Gelatin Blocking Agent

Please visit our website at www.biotium.com for information on our life science research products, including a wide selection of primary and secondary antibodies, phalloidins, lectins, and Mix-n-Stain™ antibody labeling kits featuring our bright and photostable fluorescent CF® Dyes.

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