Revised: May 29, 2018

Product Information

TrueBlack® IF Background Suppressor System (Permeabilizing)

Component	23012-T 20 assays*	23012 200 assays*
23012A TrueBlack® IF Background Suppressor (Permeabilizing)	1 mL	10 mL
23012B TrueBlack® IF Blocking Buffer (Permeabilizing)	1 mL	10 mL

*Number of assays based on two drops (~50 uL) per assay; actual number of assays may vary depending on protocol used and specimen size.

Storage and Handling

Store at 4°C. Product is stable for at least 12 months from date of receipt when stored as recommended. We recommend warming the dropper bottles at room temperature for few minutes before use for easier dispensing.

Appearance

23012A TrueBlack® IF Background Suppressor (Permeabilizing): Light blue solution. Background Suppressor may become slightly turbid at 4°C, this does not affect performance. The buffer can be warmed to room temperature or 37°C until clear.

23012B TrueBlack® IF Blocking Buffer (Permeabilizing): Colorless solution.

Product Description

The TrueBlack® Background Suppressor System is a buffer system designed for optimal blocking of non-specific staining for immunofluorescence (IF). The buffers are designed to block background from both non-specific antibody binding as well as direct interaction of fluorescent dyes on antibodies with cells or tissue sections.

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 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.

TrueBlack® Background Suppressor is designed for blocking both non-specific protein binding as well as background from charged dyes. The buffers can be used for both blocking and antibody dilution. For some antibodies, best results are obtained when the Background Suppressor is used for blocking and the Blocking Buffer is used for antibody dilution. The Blocking Buffer alone also can provide excellent results when used for both blocking and antibody dilution for conjugates that do not carry excess charge. We recommend testing the different buffers to find the combination that works best for your antibody.

The buffers in the TrueBlack® Background Suppressor System (Permeabilizing) contain non-mammalian based blocking agents plus detergent for simultaneous blocking and permeabilization for intracellular immunofluorescence. The Background Suppressor contains additional blocking agents for suppressing background from charged dyes. The buffers are provided in dropper bottles for easy dispensing.

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

Protocols

Direct intracellular immunofluorescence

1. Fix cells or tissue sections using the method recommended for your primary antibody or other conjugate.
2. Rinse samples twice with PBS.
3. Add enough TrueBlack® Background Suppressor to completely cover your sample (see Note 1).
4. Incubate at room temperature for 10 minutes or longer (see Note 2).
5. Remove the blocking buffer and add fluorescent primary antibody diluted in either Background Suppressor or Blocking Buffer (see Note 3). Phalloidins, lectins, or nuclear stains can be included in this step.
6. Incubate at room temperature for 2 hours, or at 4°C overnight, protected from light (see Note 2).
7. Rinse samples twice with PBS. Wash 3 x 5 minutes with PBS, protected from light. Alternatively, a single 30 minute wash can be done.
8. Rinse samples twice with PBS.
9. Mount samples with antifade mounting medium and image.

Indirect intracellular immunofluorescence

1. Fix cells or tissue sections using the method recommended for your primary antibody or other conjugate.
2. Rinse samples twice with PBS.
3. Add enough TrueBlack® Background Suppressor to completely cover your sample (see Note 1).
4. Incubate at room temperature for 10 minutes or longer (see Note 2).
5. Remove the blocking buffer and add primary antibody diluted in either Background Suppressor or Blocking Buffer (see Note 3).
6. Incubate with primary antibody at room temperature for 2 hours, or at 4°C overnight (see Note 2).
7. Rinse samples twice with PBS. Wash 3 x 5 minutes with PBS. Alternatively, a single 30 minute wash can be done.
8. Rinse samples twice with PBS.
9. Add secondary antibody diluted in Background Suppressor or Blocking Buffer (see Note 3). Phalloidins, lectins, or nuclear stains can be included in this step. Incubate at room temperature, protected from light, for 30 minutes to 2 hours.
10. Rinse samples twice with PBS. Wash 3 x 5 minutes with PBS, protected from light. Alternatively, a single 30 minute wash can be done.
11. Rinse samples twice with PBS.
12. Mount samples with antifade mounting medium and image.

Notes

Note 1: One drop from the dropper bottle is about 25 uL. Two drops is usually enough to cover cells in a 96-well plate, or a 2 cm^2 square tissue section.

Note 2: For tissue sections, add buffer to the section and cover with a square of Parafilm® to spread the solution over the sample, making sure there are no bubbles. Perform incubations in a humidified chamber to keep the sections from drying out.

Note 3: Either the Background Suppressor or the Blocking Buffer may be used to dilute antibodies for staining. We recommend testing each buffer to find the optimal conditions for your antibody.

Continued next page

Related Products

Catalog number	Product
23001	EverBrite™ Mounting Medium
23002	EverBrite™ Mounting Medium with DAPI
23003	EverBrite™ Hardset Mounting Medium
23004	EverBrite™ Hardset Mounting Medium with DAPI
23005	CoverGrip™ Coverslip Sealant
23007	TrueBlack® Lipofuscin Autofluorescence Quencher
40061	RedDot™2 Far-Red Nuclear Stain for dead or fixed cells
40083	NucSpot® 470 Nuclear Stain for dead or fixed cells
40081	NucSpot® Live 488 Nuclear Stain for live or fixed cells
40082	NucSpot® Live 650 Nuclear Stain for live or fixed cells
30092	MemBrite™ Fix 405/430 Cell Surface Staining Kit
30093	MemBrite™ Fix 488/515 Cell Surface Staining Kit
30094	MemBrite™ Fix 543/560 Cell Surface Staining Kit
30095	MemBrite™ Fix 568/580 Cell Surface Staining Kit
30096	MemBrite™ Fix 594/615 Cell Surface Staining Kit
30097	MemBrite™ Fix 640/660 Cell Surface Staining Kit
30098	MemBrite™ Fix 660/680 Cell Surface Staining Kit
30099	MemBrite™ Fix 680/700 Cell Surface Staining Kit
30090	CellBrite™ Fix 488 Membrane Stain
30088	CellBrite™ Fix 555 Membrane Stain
30089	CellBrite™ Fix 640 Membrane Stain
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
22020	10X Phosphate-Buffered Saline (PBS)

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.