SZABO SCANDIC

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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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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.

Note: Component A (Background Suppressor) may become turbid or form a gel at 4°C; this does not affect performance. Warm the buffer to room temperature or 37°C until clear (light blue) and completely liquid before use.

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 (Component A) contains specialized blocking agents for suppressing non-specific binding from charged dyes. Both the Background Suppressor (Component A) and Blocking Buffer (Component B) additionally contain non-mammalian based protein blocking agents plus detergent for simultaneous blocking and permeabilization for intracellular immunofluorescence. Either of the components can be used for blocking and antibody dilution steps; we recommend testing each buffer to find the combination that works best for your antibody (see Note 3 under Considerations for Staining). 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

Considerations for Staining

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.
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.
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. We have found that using the Blocking Buffer for diluting the antibody gives gives the best results. The Blocking Buffer alone can also provide excellent results when used for blocking and antibody dilution for conjugates that do not carry excess charge. If you choose to use Background Suppressor for both pre-treatment and antibody dilution, it will reduce the number of assays you can do with this kit.

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 (Cat. no. 23012A) to completely cover your sample.
4. Incubate at room temperature for 10 minutes or longer.
5. Remove the Background Suppressor (Cat. no. 23012A) and add fluorescent primary antibody diluted in Blocking Buffer (Cat. no. 23012B) (see Note 3 under Considerations for Staining). 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.
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 (Cat. no. 23012A) to completely cover your sample.
4. Incubate at room temperature for 10 minutes or longer.
5. Remove the Background Suppressor (Cat. no. 23012A) and add primary antibody diluted in Blocking Buffer (Cat. no. 23012B) (see Note 3 under Considerations for Staining).
6. Incubate with primary antibody at room temperature for 2 hours, or at 4°C overnight.
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 fluorescent secondary antibody diluted in Blocking Buffer (Cat. no. 23012B) (see Note 3 under Considerations for Staining). 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.

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
23012	TrueBlack® IF Background Suppressor System (Permeabilizing)
23013	TrueBlack® WB Blocking Buffer Kit
23014	TrueBlack® Plus Lipofuscin Autofluorescence Quencher, 40X in DMSO
23017-23019	EverBrite TrueBlack® Hardset Mounting Medium
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
30092-30099	MemBrite™ Fix Cell Surface Staining Kits
30088-30090	CellBrite™ Fix Membrane Staining Kits
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative

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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