Product Information

TrueBlack® IF Background Suppressor System (Permeabilizing)

Kits (Supplied in Dropper Bottles)

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

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

Standalone Buffer (Supplied in Screw-Cap Bottles)

Product Name	100 mL	1 L
TrueBlack® IF Blocking Buffer (Permeabilizing)	23012B-100ML	23012B-1L

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 a few minutes before use for easier dispensing.

Note: Background Suppressor (Component A) 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® IF 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 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 Cy® 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 conjugate 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® IF 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) 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 Considerations for Staining).

The TrueBlack® IF 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).

Considerations for Staining

- 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.
- 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.
- Background Suppressor or 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 Blocking Buffer for diluting the antibody gives the best results. Blocking Buffer alone can also provide excellent results when used for blocking and antibody dilution for conjugates that do not carry excess charge, such as CF® Dye conjugates. If you choose to use the same buffer for both blocking and antibody dilution, it will reduce the number of assays you can do with this kit. However, Blocking Buffer may be purchased as a standalone reagent.

Experimental 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® IF Background Suppressor (Component A) to completely cover your sample.
4. Incubate at room temperature for 10 minutes or longer.
5. Remove the Background Suppressor and add fluorescent primary antibody diluted in Blocking Buffer (Component B) (see 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® IF Background Suppressor (Component A) to completely cover your sample.
4. Incubate at room temperature for 10 minutes or longer.
5. Remove the Background Suppressor and add primary antibody diluted in Blocking Buffer (Component B) (see 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 (Component B) (see 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

Cat. No.	Product
23007, 23011	TrueBlack® Lipofuscin Autofluorescence Quencher
23014	TrueBlack® Plus Lipofuscin Autofluorescence Quencher, 40X in DMSO
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
22033	1X PBS (2L) Buffer Powder Packets
41033... 41040	NucSpot® Nuclear Stains
30131... 30140	CytoLiner™ Fixed Cell Membrane Stains
23001, 23002	EverBrite™ Mounting Medium (with or without DAPI)
23003... 23016	EverBrite™ Hardset Mounting Medium (with or without DAPI or NucSpot® 640)
23017-23019	EverBrite TrueBlack® Hardset Mounting Medium (with or without DAPI or NucSpot® 640)
23008, 23009	Drop-n-Stain EverBrite™ Mounting Medium (with or without DAPI)
22030	AntiFix™ Universal Antigen Retrieval Buffer, 10X
33000-33020	Tyramide Amplification Kits
92170... 96128	CF® Dye Tyramide
40061	RedDot™2 Far-Red Nuclear Stain, 200X in DMSO
40009... 40043	DAPI
23005	CoverGrip™ Coverslip Sealant
23023, 23024	Super^{HT} PAP Pen 2.0
23013	TrueBlack® WB Blocking Buffer Kit
22015	Fixation Buffer
22016	Permeabilization Buffer
22017	Permeabilization and Blocking Buffer (5X)
22013	Bovine Serum Albumin Fraction V
22014	Bovine Serum Albumin 30% Solution
22010	10X Fish Gelatin Blocking Agent
22011	Fish Gelatin Powder

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, Mix-n-Stain™ antibody labeling kits, and other fluorescent probes.