Revised: July 31, 2017

Product Information

Permeabilization and Blocking Buffer (5X)

Catalog Number: 22017

Unit Size: 100 mL

Color and Form: Light brown solution

Storage and Handling: Store at -20°C. Product is stable for at least 6 months from date of receipt when stored as recommended. Components of Permeabilization and Blocking Buffer (5X) may precipitate at low temperature. If precipitate is visible, warm the 5X buffer to 37°C and vortex to re-dissolve.

Product Description

Permeabilization and Blocking Buffer (5X) is a concentrated buffer for permeabilization and blocking of fixed cells for intracellular immunofluorescence. Permeabilization and Blocking Buffer also can be used to dilute primary antibodies for staining steps, and as a wash buffer.

Note: Permeabilization and Blocking Buffer is now supplied without preservative. Store the buffer at -20°C. Sodium azide can be added to the buffer before or after dilution at a final concentration of 0.02%. If azide is added, 5X buffer or 1X buffer can be stored at 4°C for at least 6 months. Sodium azide should not be used with HRP conjugates.

Note: Permeabilization and Blocking Buffer contains goat serum, and is not recommended for use with anti-goat secondary antibodies. We recommend using Fish Gelatin Blocking Buffer (catalog no. 22010) with anti-goat antibodies.

Preparation of 1X Permeabilization and Blocking Buffer

Combine one volume of 5X Permeabilization and Blocking Buffer with 4 volumes of dH_2O and mix well. Store 1X Permeabilization and Blocking Buffer at -20°C for up to 6 months. If sodium azide is added at a final concentration of 0.02%, the buffer can be stored at 4°C for at least 6 months. Discard 1X Permeabilization and Blocking Buffer if cloudy.

Immunofluorescence Protocol for Microscopy

1X Permeabilization and Blocking Buffer can be for permeabilization/blocking, antibody incubation, and washing steps in any standard immunofluorescence protocol. The protocol below is provided as a reference and may be optimized for specific applications.

1. Coverslip preparation for adherent cells

1.1 Culture cells on slide chambers or sterile glass coverslips (with poly-L-lysine coating if cells do not adhere well, see below). We recommend 18 x 18 mm square coverslips in 6-well plates or 4-well chamber slides.
1.2 Allow cells to adhere and culture or treat as desired.

2. Coverslip preparation for non-adherent cells

2.1 Coat coverslips with 0.01% poly-L-lysine solution for 10 minutes at room temperature.
2.2 Aspirate the poly-L-lysine solution and allow coverslips to dry completely.
2.3 Centrifuge cells in medium and resuspend in PBS. Transfer cells to coverslips.
2.4 Incubate for 30-60 minutes. Check for adherence under microscope.

3. Fixation and Staining

3.1 Rinse cells three times in PBS to remove culture medium.
3.2 Incubate cells in Fixation Buffer (catalog no. 22015), 15 minutes at room temperature. Alternatively, cells can be fixed in chilled (4°C) Fixation Buffer on ice for 30 minutes. Other fixatives also can be used.
3.3 Rinse twice with PBS to remove traces of fixative.
3.4 Incubate with 1X Permeabilization and Blocking Buffer, 30 minutes at room temperature.
3.5 Dilute primary antibody in 1X Permeabilization and Blocking Buffer to the concentration recommended by the antibody supplier. A negative control with primary antibody omitted is recommended to assess background. Overlay enough diluted antibody solution to completely cover cells. Parafilm® squares can be overlaid on coverslips to evenly spread a small volume (~100 uL) of antibody solution over the surface. Keep slips in a humidified chamber to avoid evaporation. Incubate 2 hours at room temperature, or overnight at 4°C.
3.6 Wash three times with PBS, 5 minutes each wash. 1X Permeabilization and Blocking Buffer can be used instead of PBS for a more stringent wash.
3.7 Dilute fluorescent secondary antibody in dilution buffer and incubate for 30 minutes to 2 hours at room temperature. IgG conjugates can be used at 1-10 µg/mL for most applications. Fluorescent nuclear stains or phalloidins can be included at this step.
3.8 Wash three times with PBS, 5 minutes each wash. 1X Permeabilization and Blocking Buffer can be used instead of PBS for a more stringent wash.
3.9 Mount coverslips using anti-fade mounting media, such as EverBrite™ Mounting Medium (see related products), or add enough mounting medium to wells or chambers to completely cover cells. Seal coverslip edges with clear nail polish or CoverGrip™ coverslip sealant (catalog no. 23005).
3.10 Store slides in the dark at 4°C.

Tips and Hints:

1. No signal or weak fluorescence intensity may suggest the following: (a) insufficient antibody is present for detection, (b) intracellular target was not accessible, (c) excitation sources are not aligned, (d) target protein is not present or expressed at low levels, (e) fluorochrome has faded, and/or (f) primary and secondary antibodies are not compatible.
2. High fluorescence background may suggest the following: (a) antibody concentration is too high, (b) excess antibody was not washed away efficiently, and/or (c) blocking was inadequate. Increase antibody dilution and washes.

Related Products

Cat.#	Product Name	Unit Size
23006	Flow Cytometry Fixation/Permeabilization Kit	50 assays
22015	Fixation Buffer	100 mL
22016	Permeabilization Buffer	100 mL
23001	EverBrite™ Mounting Medium	10 mL
23002	EverBrite™ Mounting Medium with DAPI	10 mL
23003	EverBrite™ Hardset Mounting Medium	10 mL
23004	EverBrite™ Hardset Mounting Medium + DAPI	10 mL
23005	CoverGrip™ Coverslip Sealant	15 mL
22010	10% Fish Gelatin Blocking Buffer	100 mL
22011	Fish Gelatin Powder	2 x 50 g
22014	30% Bovine Serum Albumin Solution	100 mL
22012	Dry Milk Powder	4 x 25 g
22002	Tween®-20	50 mL

Please visit www.biotium.com to view our full selection of products featuring bright and photostable fluorescent CF™ dyes, including secondary antibodies and Mix-n-Stain™ antibody labeling kits.