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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
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Product Information

Flow Cytometry Fixation/Permeabilization Kit

Catalog Number: 23006

Unit Size: 50 tests

Kit Contents

Component	Size
99970: Flow Cytometry Fixation Buffer	1 X 5 mL
99971: Flow Cytometry Permeabilization Buffer	1 X 5 mL

Storage and Handling

Store at room temperature. Do not freeze. Buffers are stable for 6 months from the date of receipt.

Product Description

Flow Cytometry Fixation/Permeabilization Kit contains optimally formulated buffers for fixation and permeabilization of cells for immunofluorescence staining of intracellular antigens for analysis by flow cytometry.

Sample data

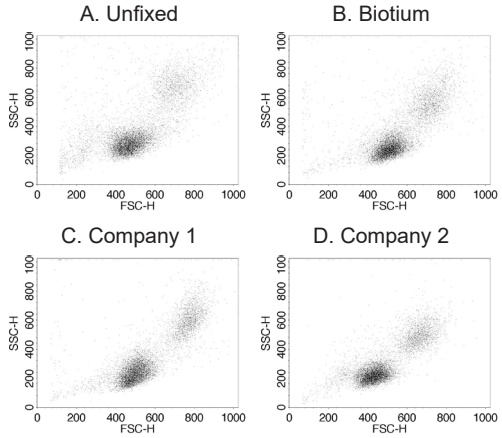

Figure 1. Comparison of Biotium's Flow Cytometry Fixation/Permeabilization Kit with leading competitors' fixation/permeabilization kits. Primary human PBMCs were left unfixed (A) or fixed and permeabilized according to kit manufacturer's protocols (B-D) and analyzed on a BD FACSCalibur flow cytometer for forward/side scatter profiles.

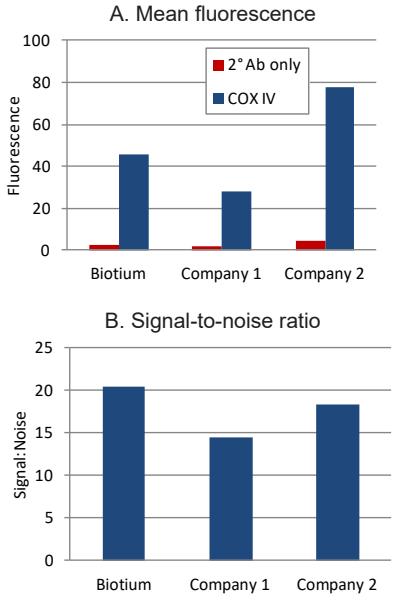

Figure 2. Comparison of immunofluorescence staining for an intracellular antigen using Biotium's Flow Cytometry Fixation/Permeabilization Kit compared to leading competitors' kits. Jurkat cells were fixed and permeabilized according to kit protocols, stained with rabbit anti-COXIV antibody followed by CF®488A-conjugated goat anti-rabbit secondary antibody, and analyzed on a BD FACSCalibur flow cytometer in channel FL1. (A) Fluorescence signal with and without primary antibody. Bars represent the geometric mean fluorescence of the cell populations. (B) Signal-to-noise ratio.

Experimental Protocols

Materials required but not provided

- 1X phosphate buffered saline (PBS)
- Wash buffer: PBS + 2% bovine serum or goat serum (optional: add 0.1% sodium azide for long term storage at 4°C)

Fluorescent staining for flow cytometry

Note: Any incubations performed after the addition of fluorescently-labeled primary or secondary antibodies need to be performed in the dark.

1. Pellet cells by centrifuging at 350 x g for 5 minutes and resuspend in PBS at a density of 10^7 cells/mL.
2. Aliquot 100 uL of cell suspension (10^6 cells) per tube into 12 x 75 mm polypropylene flow cytometry tubes.
3. Optional: Staining for surface antigens.
   a. Dilute the appropriate amount of surface antigen antibody(s) in PBS.
   b. Add ~100 uL of the antibody solution to each tube.
   c. Incubate for 15 minutes at room temperature.
   d. Wash cells twice with 1 mL PBS as described in step 1.
   e. Resuspend cells in 100 uL PBS.
4. Add 100 uL of Fixation Buffer to each sample and vortex gently to mix.
5. Incubate for 20 minutes at room temperature.
6. Centrifuge for 5 minutes at 350 x g. Wash cells twice by resuspending in 1 mL wash buffer (see materials required but not provided), then centrifuge for 5 minutes at 350 x g, and gently pour off supernatant.
7. Dilute the appropriate amount of primary antibody in permeabilization buffer and vortex gently to mix.

8. Add ~100 uL of the primary antibody solution to each tube.
9. Incubate samples at room temperature for 30 minutes.
10. Wash cells twice with wash buffer as described in step 6.

    Note: If staining with fluorescently-labeled primary antibodies, add 1 mL wash buffer and analyze by flow cytometry. If staining with unconjugated primary antibodies and fluorescently-labeled secondary antibodies, proceed to step 11.
11. Dilute the appropriate amount of fluorescent secondary antibody in wash buffer and vortex gently to mix.
12. Add ~100 uL of the secondary antibody solution to each tube.
13. Incubate for 30 minutes at room temperature in the dark.
14. Wash cells twice with wash buffer as described in step 6.
15. Resuspend cell pellet in 1 mL wash buffer and analyze by flow cytometry.

Related Products

Catalog number	Product
30050	ViaFluor® CFSE Cell Proliferation Kit
30068	ViaFluor® 405 SE Cell Proliferation Kit
30086	ViaFluor® 488 SE Cell Proliferation Kit
32002-32013	Live-or-Dye™ Fixable Viability Staining Kits
22020	10X Phosphate-Buffered Saline (PBS)
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
22015	Fixation Buffer
22016	Permeabilization Buffer
22017	Permeabilization and Blocking Buffer (5X)
22010	10X Fish Gelatin Blocking Buffer
22011	Fish Gelatin Powder
22014	30% Bovine Serum Albumin Solution
22012	Dry Milk Powder
22003	Mini Cell Scrapers
22002	Tween® 20

Please visit www.biotium.com to view our full selection of products featuring bright and photostable fluorescent CF® dyes, including primary and secondary antibodies and Mix-n-Stain™ antibody labeling kits. Biotium also offers a variety of apoptosis and cell viability assays for flow cytometry analysis, including mitochondrial membrane potential dyes, fluorescent Annexin V conjugates, and NucView®488 Caspase-3 Substrate for live cells.