Biotium
Glowing Products for Science™

3159 Corporate Place
Hayward, CA 94545
www.biotium.com

Revised: June 26, 2012

Product Information

Flow Cytometry Fixation/Permeabilization Kit

Catalog Number: 23006

Unit Size: 50 tests

Components:
Fixation Buffer, 5 mL
Permeabilization Buffer, 5 mL

Color and Form: Colorless solutions

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

Warning: Fixation Buffer contains formaldehyde, which is toxic by ingestion, inhalation, and contact with skin, and is a suspected carcinogen. Avoid contact with skin, eyes, and clothing. Dispose as toxic waste. Permeabilization Buffer contains 0.1% sodium azide as a preservative.

Product Description

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

Sample data

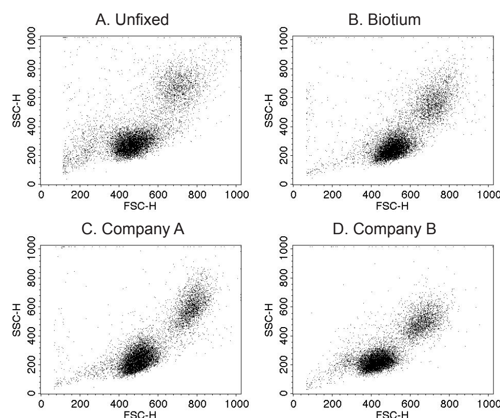

Figure 1. Comparison of Biotium's Flow Cytometry Fixation/Permeabilization Kit with leading competitors' fixation/permeabilization kits. Primary human PBMC were left unfixed (A) or fixed and permeabilized according to kit manufacturer's protocols (B-D) and analyzed on a BD FACSCalibur flow cytometer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

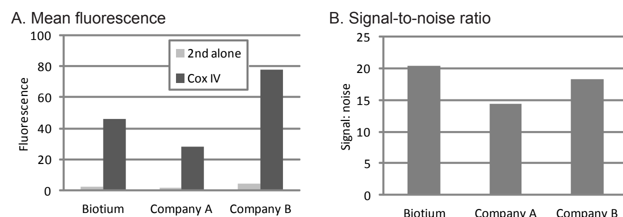

Figure 2. Comparison of immunofluorescence staining for an intracellular antigen using Biotium's Flow Cytometry Fixation/Permeabilization Kit compared to leading competitors' kits. Jurkat cells were fixed and permeabilized according to kit protocols and stained with rabbit anti-COXIV antibody followed 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.

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)

Protocol for intracellular staining for flow cytometry

1. Pellet cells by centrifuging at 350 x *g* for 5 minutes. Wash cells twice in PBS. To wash cells, resuspend the cell pellet in PBS, centrifuge at 350 x *g* for 5 minutes, and gently pour off supernatant. Resuspend cells 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. Staining for surface antigens can be performed at this point:
   a. Add the appropriate antibodies to cells in PBS.
   b. Incubate for 15 minutes at room temperature in the dark.
   c. Wash cells twice with 3 mL PBS as described in step 1.
   d. Resuspend cells in 100 uL PBS.

4. Add 100 uL of Fixation Buffer to each tube 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 in wash buffer (see materials required but not provided above). To wash cells, resuspend cell pellet in 3 mL wash buffer, centrifuge for 5 minutes at 350 x *g*, and gently pour off supernatant.

7. Add 100 uL of Permeabilization Buffer to each tube. Add primary antibodies to the permeabilization buffer at the recommended concentrations, and vortex gently to mix. A negative control omitting primary antibodies (or using isotype controls) is recommended to measure background.

8. Incubate at room temperature for 30 minutes. If staining with fluorescently-labeled primary antibodies, incubate in the dark.

9. Wash cells twice with wash buffer (see step 6).

10. If staining with fluorescently-labeled primary antibodies, add 1 mL wash medium and analyze by flow cytometry. If staining with unconjugated primary antibodies and fluorescently-labeled secondary antibodies, proceed to step 11.

11. Resuspend the cells in the residual wash buffer remaining in the tube after step 9 (~100 uL). Add fluorescent secondary antibody conjugates at the recommended concentrations and vortex gently to mix.

12. Incubate for 30 minutes at room temperature in the dark.

13. Wash cells twice with wash buffer (see step 6).

14. Resuspend cell pellet in 1 mL wash buffer and analyze by flow cytometry.

Flow Cytometry Fixation/Permeabilization Kit

Related Products

Cat.#	Product Name	Unit Size
30069	AccuEasy™ Flow Cytometry Kit	1 kit
22015	Fixation Buffer	100 mL
22016	Permeabilization Buffer	100 mL
22017	Permeabilization and Blocking Buffer	100 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. 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.

CF, Mix-n-Stain, and NucView are trademarks of Biotium. Tween is a registered trademark of Uniqema Americas LLC.

Disclaimer: Materials from Biotium are sold for research use only, and are not intended for food, drug, household, or cosmetic use. Biotium is not liable for any damage resulting from handling or contact with this product.