ab314689 – Flow Cytometry Fixation & Permeabilization Kit

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.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab314689

Storage and Stability:

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

Materials Supplied

Item	Quantity	Storage Condition
Fixation Buffer	5 mL	Room Temperature
Permeabilization Buffer	5 mL	Room Temperature

Experimental Protocols

Materials required but not provided:

- 1X phosphate buffered saline (PBS) (ab285410)
- 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

Δ Note: *Any incubations performed after the addition of fluorescently labelled 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. Coat coverslips with 0.01% poly-L-lysine solution for 10 minutes at room temperature.

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.

Δ Note: *If staining with fluorescently labelled primary antibodies, add 1 mL wash buffer and analyze by flow cytometry. If staining with unconjugated primary antibodies and fluorescently labelled 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.

For technical support contact information, visit: www.abcam.com/contactus

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