ab185917

Cell Fixation & Permeabilization Kit

Instructions for Use

For the Fixation and Permeabilization of Human Biological fluids prior to Flow Cytometry

This product is for research use only and is not intended for diagnostic use.

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1. Introduction

Abcam's Cell Fixation and Permeabilization Kit (ab185917) is intended for first fixing cells in suspension and then permeabilizing the cell membranes. This gives antibodies access to intracellular structures and leaves the morphological scatter characteristics of cells intact. The specific formulation reduces background staining and allows the simultaneous addition of the permeabilization medium and fluorochrome labeled antibodies.

Flow cytometric analysis, with monoclonal antibodies, has so far been restricted to cell surface molecules. Intracellular structures such as cytoplasmic or nuclear enzymes, oncoproteins, cytokines, immunoglobulins etc. have been largely excluded from such studies. Also excluded from flow cytometric studies were cytoplasmic localizations of well-established membrane molecules such as CD3 and CD22 which, in their cytoplasmic form, are the most reliable lineage markers in undifferentiated leukemia. Abcam's Cell Fixation and Permeabilization Kit (ab185917) makes flow cytometric analysis of intracellular antigens as easy as the study of surface antigens. The only prerequisite is the availability of suitable antibody conjugates: most monoclonal antibody conjugates can be use although some determinants are sensitive to the fixation step involved.

This and the optimal fixation time have to be tested for each reagent.

2. Kit Contents

Components	50 Tests	200Tests
Fixation Medium (Reagent A)	5 mL	4 x 5 mL
Permeabilization Medium (Reagent B)	5 mL	4 x 5 mL

3. Storage and Handling

Kits should be stored and used at room temperature. Do not freeze.

Do not use reagents if a precipitate forms or discoloration occurs.

4. Additional Materials Required

- PBS
- Conjugated monoclonal antibody
- Centrifuge
- IgG preparation and human serum albumin.

5. Samples

- Biological fluids (blood, bone marrow and others) must be collected under sterile conditions
- Anticoagulation with EDTA or heparin is recommended.
- The samples should be stored at room temperature and should be processed and analysed within 24 hours.
- Samples with a high number of non-viable cells might cause false results, it is suggested that cell viability is determined (e.g. with propidium iodide).

6. Permeabilization and Staining Protocol

1. For each sample to be analysed add 50 µL of whole blood, bone marrow, mononuclear cells, any other primary cells of interest, or cultured cells in suspension to a 5 mL tube.
2. Add 100 µL of Reagent A.
3. Incubate for 15 minutes at room temperature.
4. Add 5 mL of PBS and centrifuge cells for 5 minutes at 300g.
5. Remove the supernatant and add 100 µL of Reagent B to cell pellet with an appropriate volume of a conjugated or unconjugated primary antibody (refer to manufacturer's datasheet).
6. Vortex at low speed for 1-2 seconds.
7. Incubate for 15 minutes at room temperature.
8. Wash cells with PBS and centrifuge for 5 minutes at 300 g.

If a conjugated primary antibody was used for staining:

9. Remove the supernatant and resuspend cells in sheath fluid for immediate analysis or resuspend cells in 0.5 mL 1% formaldehyde and store them at 2-8°C in the dark. Analyse the fixed cells within 24 hours.

If an unconjugated primary antibody was used for staining:

10. Remove the supernatant and add 100 µL of Reagent B to cell pellet with an appropriate volume of a fluorochrome-labelled secondary antibody (refer to manufacturer's datasheet).

11. Vortex at low speed for 1-2 seconds
12. Incubate for 15 minutes at room temperature.
13. Wash cells with PBS and centrifuge for 5 minutes at 300 g
14. Remove the supernatant and resuspend cells in sheath fluid for immediate analysis or resuspend cells in 0.5 mL 1% formaldehyde and store them at 2-8°C in the dark. Analyse the fixed cells within 24 hours.

Technical Support

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