ab314680 – Fixation Buffer (Flow Cytometry)

Fixation Buffer is a ready-to-use formaldehyde-based fixative for immunofluorescence microscopy or flow cytometry. Fixation Buffer can be used in any standard cell fixation protocol.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab314680

Storage and Stability:

Store at room temperature. Do not freeze. Stable for at least 6 months from the date of receipt. Discard if cloudy. 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.

Materials Supplied

Item	Quantity	Storage Condition
Fixation Buffer	100 mL	Room Temperature

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 (ab314680) 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 PBS + 2% bovine serum or goat serum. 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 (ab314681) to each tube. Add primary antibodies to the permeabilization buffer at the antibody suppliers' 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 labelled primary antibodies, incubate in the dark.

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

10. 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 next step.

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 suppliers' 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.

Immunofluorescence Protocol for Microscopy

Δ Note: *Because formaldehyde-based fixatives can compromise plasma membrane integrity, fixation at 4°C is recommended for staining of surface antigens in non-permeabilized cells.*

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, 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. Film 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 or add enough mounting medium to wells or chambers to completely cover cells. Seal coverslip edges e.g., with clear nail polish.

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.

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

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