

Applications	Reactivity	Sensitivity	Isotype
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F	H M Mk	Endogenous	Rabbit IgG
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Applications Key: F=Flow Cytometry

Reactivity Key: H=Human M=Mouse Mk=Monkey

Species cross-reactivity is determined by western blot. Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Protocols

Flow

Flow Cytometry Protocol

A. Solutions and Reagents

NOTE: Prepare solutions with purified water.

1. **1X Phosphate Buffered Saline (PBS):** Dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na₂HPO₄ and 0.24 g KH₂PO₄ in 800 ml dH₂O. Adjust the pH to 7.4 with HCl and the volume to 1 L. Store at room temperature.
2. **Formaldehyde** (methanol free).
3. **100% Methanol**
4. **Incubation Buffer:** Dissolve 0.5 g bovine serum albumin (BSA) in 100 ml 1X PBS. Store at 4 °C.

B. Fixation

1. Collect cells by centrifugation and aspirate supernatant.
2. Resuspend cells briefly in 0.5–1 ml PBS. Add formaldehyde to a final concentration of 2–4% formaldehyde.
3. Fix for 10 min at 37 °C.
4. Chill tubes on ice for 1 min.
5. For extracellular staining with antibodies that do not require permeabilization, proceed to Section D, Step 1 or store cells in PBS with 0.1% sodium azide at 4 °C; for intracellular staining, proceed to permeabilization (Section C, Step 1).

C. Permeabilization

1. Permeabilize cells by adding ice-cold 100% methanol slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol. Alternatively, to remove fix prior to permeabilization, pellet cells by centrifugation and resuspend in 90% methanol.
2. Incubate 30 min on ice.
3. Proceed with immunostaining (Section D, Step 1) or store cells at –20 °C in 90% methanol.

D. Immunostaining

NOTE: Account for isotype matched controls for monoclonal antibodies or species matched IgG for polyclonal antibodies. Count cells using a hemocytometer or alternative method.

1. Aliquot 0.5–1x10⁶ cells into each assay tube (by volume).
2. Add 2–3 ml Incubation Buffer to each tube and rinse by centrifugation. Repeat.
3. Resuspend cells in 100 µl Incubation Buffer per assay tube.
4. Block in Incubation Buffer for 10 min at room temperature.

5. Add the unconjugated, biotinylated, or fluorochrome-conjugated primary antibody at the appropriate dilution to the assay tubes (see individual antibody datasheet for the appropriate dilution).
6. Incubate for 1 hr at room temperature.
7. Rinse as before in Incubation Buffer by centrifugation.
8. If using a fluorochrome-conjugated primary antibody, resuspend cells in 0.5 ml PBS and analyze on flow cytometer; for unconjugated or biotinylated primary antibodies, proceed to immunostaining (Section D, Step 9).
9. Resuspend cells in fluorochrome-conjugated secondary antibody or fluorochrome-conjugated avidin, diluted in Incubation Buffer at the recommended dilution.
10. Incubate for 30 min at room temperature.
11. Rinse as before in Incubation Buffer by centrifugation.
12. Resuspend cells in 0.5 ml PBS and analyze on flow cytometer; alternatively, for DNA staining, proceed to optional DNA stain (Section E, Step 1).

E. Optional DNA Stain

1. Resuspend cells in 0.5 ml of DNA dye (e.g. Propidium Iodide (PI)/RNase Staining Solution [#4087](#)).
2. Incubate for at least 5 min at room temperature.
3. Analyze cells in DNA stain on flow cytometer.

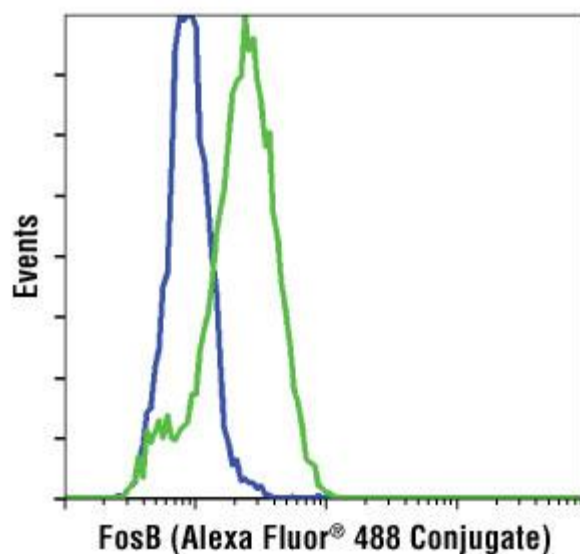
Specificity / Sensitivity

FosB (5G4) Rabbit mAb (Alexa Fluor® 488 Conjugate) detects endogenous levels of total FosB protein (both FosB and FosB2 isoforms). The antibody does not cross-react with other Fos proteins, including c-fos, FRA1 and FRA2.

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to the sequence of human FosB. The antibody was conjugated to Alexa Fluor® 488 under optimal conditions with an F/P ratio of 2-6.

Flow Cytometry



Flow cytometric analysis of HeLa cells, untreated (blue) or treated with TPA (green), using FosB (5G4) Rabbit mAb (Alexa Fluor® 488 Conjugate).

Description

This Cell Signaling Technology antibody is conjugated to Alexa Fluor® 488 fluorescent dye and tested in-house for direct flow cytometric analysis of human cells. The unconjugated antibody FosB (5G4) Rabbit mAb #2251 reacts with human, mouse and rat FosB protein. CST expects that FosB (5G4) Rabbit mAb (Alexa Fluor® 488 Conjugate) will also recognize FosB in these species.