

FosB (5G4) Rabbit mAb (Alexa Fluor® 488 Conjugate)

✓ 500 µl
(50 tests)

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This product is for *in vitro* research use only and is not intended for use in humans or animals.
This product is not intended for use as a therapeutic or in diagnostic procedures.

Entrez-Gene ID #2354
Swiss-Prot Acc. #P53539

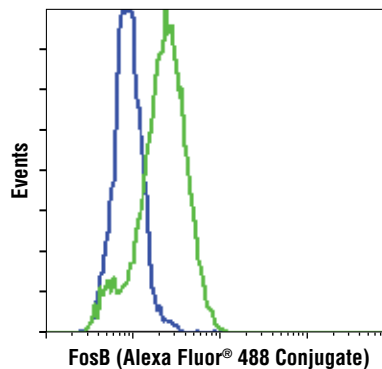
Applications	Species Cross-Reactivity*	Isotype
F Endogenous	H, M, R	Rabbit IgG

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.

Background: The Fos family of nuclear oncogenes includes c-Fos, FosB, fos-related antigen 1 (FRA1) and fos-related antigen 2 (FRA2) (1). While most Fos proteins exist as a single isoform, the FosB protein exists as two isoforms: full-length FosB and a shorter form FosB2/SF that lacks the carboxy-terminal 101 amino acids (1,2). The expression of Fos proteins is rapidly and transiently induced by a variety of extracellular stimuli, including growth factors, cytokines, neurotransmitters, polypeptide hormones and stress. Fos proteins dimerize with Jun proteins (c-Jun, JunB and JunD) to form Activator Protein-1 (AP-1), a transcription factor that binds to TRE/AP-1 elements and activates transcription. Fos and Jun proteins contain the leucine-zipper motif that mediates dimerization and an adjacent basic domain that binds to DNA. The various Fos/Jun heterodimers differ in their ability to transactivate AP-1 dependent genes. In addition to increased expression, phosphorylation of FosB and c-Fos by ERK1/2 in response to extracellular stimuli may further increase transcriptional activity (4). Expression of FosB and c-Fos in quiescent fibroblasts after growth factor stimulation is immediate, but very short-lived, with protein levels dissipating after several hours (5). However, FRA1 and FRA2 expression persists longer and appreciable levels can be detected in asynchronously growing cells (6). Deregulated expression of c-Fos, FosB, or FRA2 can result in neoplastic cellular transformation; however, FosB2 lacks the ability to transform cells (2,3).

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

Directions for Use: Add 10 µl of the conjugated antibody to 500,000 cells in 90 µl PBS/0.5% BSA. See protocol for more details.

Background References:

- (1) Tulchinsky, E. (2000) *Histol. Histopathol.* 15, 921–928.
- (2) Dobrzanski, P. et al. (1991) *Mol. Cell. Biol.* 11, 5470–5478.
- (3) Nakabeppu, Y. and Nathans, D. (1991) *Cell* 64, 751–759.
- (4) Rosenberger, S.F. et al. (1999) *J. Biol. Chem.* 274, 1124–1130.
- (5) Kovary, K. and Bravo, R. (1991) *Mol. Cell. Biol.* 11, 2451–2459.
- (6) Kovary, K. and Bravo, R. (1992) *Mol. Cell. Biol.* 12, 5015–5023

Storage: Supplied in PBS (pH 7.2), less than 0.1% sodium azide, 2 mg/ml BSA. Store at 4°C. Protect from light. *Do not freeze.*

***Species cross-reactivity other than human is predicted by Western analysis using the unconjugated antibody.**

Recommended Antibody Dilutions:

Flow Cytometry 1:10

Companion Products:

FosB (5G4) Rabbit mAb #2251
FosB Antibody #2263
c-Jun (60A8) Rabbit mAb #9165
Phospho-c-Jun (Ser63) II Antibody #9261
FosB Blocking Peptide #1042

The Alexa Fluor® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc., for research use only, except for use in combination with DNA microarrays. The Alexa Fluor® dyes (except for Alexa Fluor® 430 dye) are covered by pending and issued patents.

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Flow Cytometry Protocol for Intracellular Staining Using Conjugated Primary Antibodies

A Solutions and Reagents

1. **1X Phosphate Buffered Saline (PBS):** Dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4 in 800 ml distilled water (dH_2O). Adjust the pH to 7.4 with HCl and the volume to 1 liter. Store at room temperature.
2. Formaldehyde (methanol free)
3. **Incubation Buffer:** Dissolve 0.5 g bovine serum albumin (BSA) in 100ml 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 minutes at 37°C.
4. Chill tubes on ice for 1 minute.

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 minutes on ice.
3. Proceed with staining or store cells at -20°C in 90% methanol.

D Staining Using Conjugated Primary Antibodies

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

1. Aliquot 5×10^5 cells into each assay tube (by volume).
2. Add 2-3 ml Incubation Buffer to each tube and rinse by centrifugation.
3. Resuspend cells in 90 μl Incubation Buffer per assay tube.
4. Block in Incubation Buffer for 10 minutes at room temperature.
5. Add 10 μl of conjugated antibody to the assay tubes.
6. Incubate for 30-60 minutes, in the dark at room temperature.
7. Rinse as before in Incubation Buffer by centrifugation.
8. Resuspend cells in 0.5 ml PBS and analyze on flow cytometer.