Store at -20°C

FosB (5G4) Rabbit mAb

#2251

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Entrez-Gene ID #2354
UniProt ID #P53539

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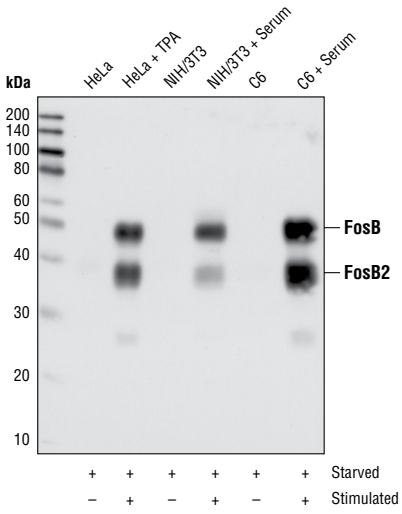
Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P, IF-IC, ChIP, F Endogenous	H, M, R	38 kDa FosB2 48 kDa FosB	Rabbit IgG**

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 (Delta FosB), that lacks the carboxy-terminal 101 amino acids (1-3). 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 Fos proteins by Erk kinases in response to extracellular stimuli may further increase transcriptional activity (4-6). Phosphorylation of c-Fos at Ser32 and Thr232 by Erk5 increases protein stability and nuclear localization (5). Phosphorylation of FRA1 at Ser252 and Ser265 by Erk1/2 increases protein stability and leads to overexpression of FRA1 in cancer cells (6). Following growth factor stimulation, expression of FosB and c-Fos in quiescent fibroblasts is immediate, but very short-lived, with protein levels dissipating after several hours (7). FRA1 and FRA2 expression persists longer, and appreciable levels can be detected in asynchronously growing cells (8). Deregulated expression of c-Fos, FosB, or FRA2 can result in neoplastic cellular transformation; however, Delta FosB lacks the ability to transform cells (2,3).

Specificity/Sensitivity: FosB (5G4) Rabbit mAb 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.



Western blot analysis of extracts from HeLa cells serum-starved overnight and TPA-stimulated for 4 hours, or NIH/3T3 cells and C6 cells serum-starved overnight and serum-stimulated for 4 hours, using FosB (5G4) Rabbit mAb.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibody.*

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:50
Immunohistochemistry (Paraffin)	1:50†
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114

†*Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.*

Immunofluorescence (IF-IC)	1:800
Chromatin IP	1:50
Flow Cytometry	1:200

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com

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) Sasaki, T. et al. (2006) *Mol. Cell* 24, 63-75.

(6) Basbous, J. et al. (2007) *Mol. Cell. Biol.* 27, 3936-3950.

(7) Kovary, K. and Bravo, R. (1991) *Mol. Cell. Biol.* 11, 2451-2459.

(8) Kovary, K. and Bravo, R. (1992) *Mol. Cell. Biol.* 12, 5015-5023.

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

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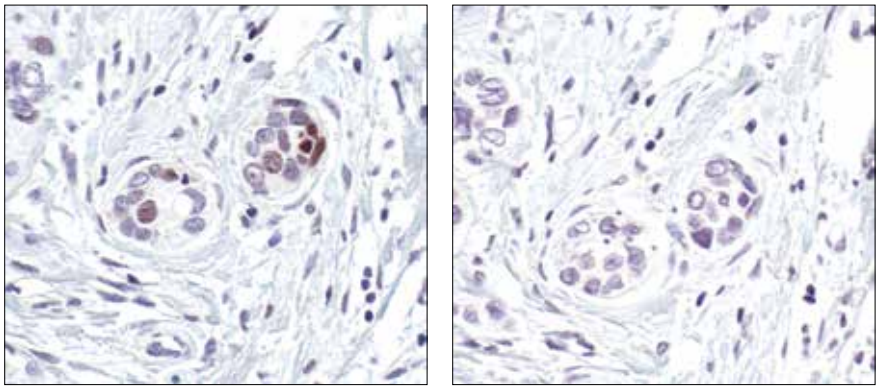
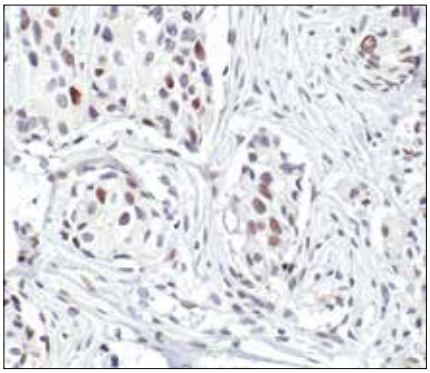
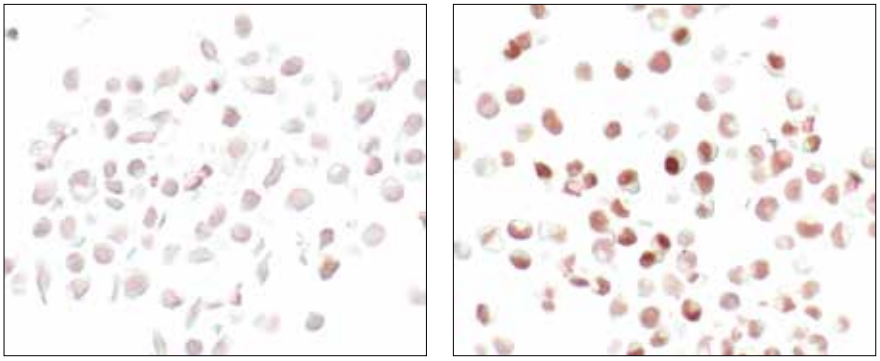
Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



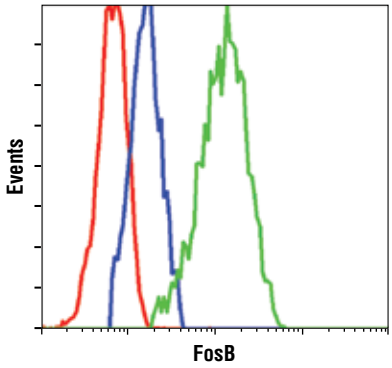
Immunohistochemical analysis of paraffin-embedded human breast carcinoma, using FosB (5G4) Rabbit mAb in the presence of control peptide (left) or FosB Blocking Peptide #1042 (right).



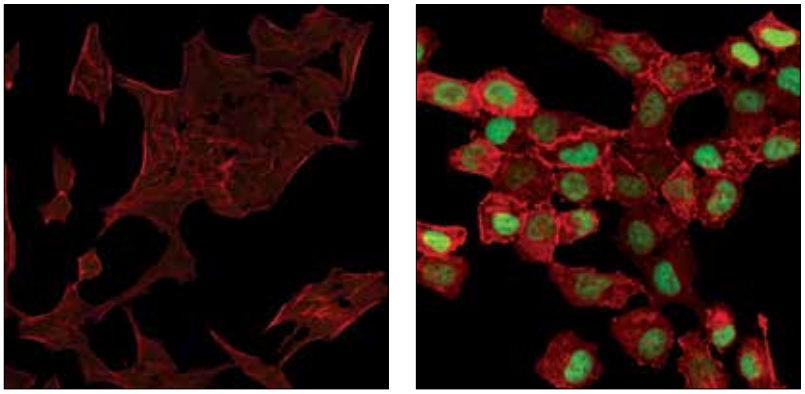
Immunohistochemical analysis of paraffin-embedded human breast carcinoma, using FosB (5G4) Rabbit mAb.



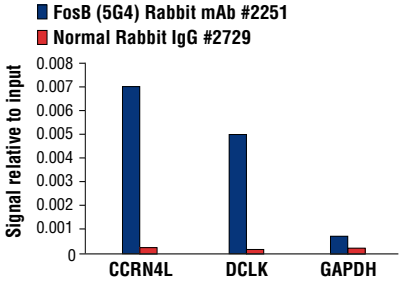
Immunohistochemical analysis of paraffin-embedded HeLa cells control (left) or PMA-treated (right), using FosB (5G4) Rabbit mAb.



Flow cytometric analysis of Hela cells, untreated (blue) or TPA treated (green), using FosB (5G4) Rabbit mAb compared to a nonspecific negative control antibody (red).



Confocal immunofluorescent analysis of HeLa cells either serum-starved (left) or TPA-treated (right) and labeled with FosB (5G4) Rabbit mAb (green). Actin filaments have been labeled with Alexa Fluor® 555 phalloidin (red).



Chromatin immunoprecipitations were performed with cross-linked chromatin from 4 x 10^6 PC-12 cells starved overnight and treated with β-NGF #5221 (50 ng/ml) for 2 hr and either 10 µl of FosB (5G4) Rabbit mAb or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR SimpleChIP® Rat CCRN4L Promoter Primers #7983, rat DCLK1 promoter primers, and SimpleChIP® Rat GAPDH Promoter Primers #7964. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.