Fos Family Antibody Sampler Kit

#8333 Store at -20°C

☑ 1 Kit
(5 x 20 µl)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 06/16

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
c-Fos Antibody	4384	20 µl	62 kDa	Rabbit IgG
FosB (5G4) Rabbit mAb	2251	20 µl	38, 48 kDa	Rabbit IgG
Phospho-c-Fos (Ser32) (D82C12) XP® Rabbit mAb	5348	20 µl	62 kDa	Rabbit IgG
FRA1 (D80B4) Rabbit mAb	5281	20 µl	40 kDa	Rabbit IgG
Phospho-FRA1 (Ser265) (D22B1) Rabbit mAb	5841	20 µl	40 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions, and additional application protocols.

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 antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

U.S. Patent No. 5,675,063

Description: The Fos Family Antibody Sampler Kit provides an economical means to evaluate the Fos family of transcription factors. The kit includes enough primary antibody to perform two western blot experiments with each primary antibody.

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 (ΔFosB), 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 on Ser32 and Thr232 by Erk5 increases protein stability and nuclear localization (5). Phosphorylation of FRA1 on 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, FosB2 lacks the ability to transform cells (2,3).

Specificity/Sensitivity: Each antibody in the Fos Family Antibody Sampler Kit recognizes endogenous levels of the specific target protein. FosB (5G4) Rabbit mAb detects both FosB and FosB2 isoforms. Phospho-FRA1 (Ser265) (D22B1) Rabbit mAb recognizes endogenous levels of FRA1 protein only when phosphorylated at Ser265. This antibody may also cross-react with phospho-FRA2, but does not cross-react with phospho-c-Fos or phospho-FosB.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser32 of human c-Fos, Ser265 of human FRA1 protein or with a synthetic peptide derived from human FosB, or FRA1. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to amino acids near the carboxy-terminus of human c-Fos protein. Antibodies are purified by protein A and peptide affinity chromatography.

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.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **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.

#8333

Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.