#9914 Store at -20°C

Phospho-Stat Antibody Sampler Kit

☑ 1 Kit
(6 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.	Source
Phospho-Stat1 (Tyr701) (D4A7) Rabbit mAb	7649	20 µl	84, 91 kDa	Rabbit IgG
Phospho-Stat2 (Tyr690) Antibody	4441	20 µl	113 kDa	Rabbit IgG
Phospho-Stat3 (Tyr705) (D3A7) XP® Rabbit mAb	9145	20 µl	79, 86 kDa	Rabbit IgG
Phospho-Stat3 (Ser727) Antibody	9134	20 µl	86 kDa	Rabbit IgG
Phospho-Stat5 (Tyr694) (D47E7) XP® Rabbit mAb	4322	20 µl	90 kDa	Rabbit IgG
Phospho-Stat6 (Tyr641) Antibody	9361	20 µl	110 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.

Description: The Phospho-Stat Pathway Sampler Kit provides an economical means to evaluate the activation status of Stat molecules, including the phosphorylation of Stat1 at Tyr701, Stat2 at Tyr690, Stat3 at Tyr705/Ser727, Stat5 at Tyr694 and Stat6 at Tyr641. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: Jaks (Janus Kinases) and Stats (Signal Transducers and Activators of Transcription) are utilized by receptors for a wide variety of ligands including cytokines, hormones, growth factors and neurotransmitters. Jaks, activated via autophosphorylation following ligand-induced receptor aggregation, phosphorylate tyrosine residues on associated receptors, Stat molecules and other downstream signaling proteins (1,2). The phosphorylation of Stat proteins at conserved tyrosine residues activates SH2-mediated dimerization followed rapidly by nuclear translocation. Stat dimers bind to IRE (interferon response element) and GAS (gamma interferon-activated sequence) DNA elements, resulting in the transcriptional regulation of downstream genes (1,2). The remarkable range and specificity of responses regulated by the Stats is determined in part by the tissue-specific expression of different cytokine receptors, Jaks and Stats (2,3), and by the combinatorial coupling of various Stat members to different receptors. Serine phosphorylation in the carboxy-terminal transcriptional activation domain has been shown to regulate the function of Stat1, -2, -3, -4 and -5 (1). Phosphorylation of Stat3 at Ser727 via MAPK or mTOR pathways is required for optimal transcriptional activation in response to growth factors and cytokines including IFN-gamma and CNTF (4,5). Jak/Stat pathways also play important roles in oncogenesis, tumor progression, angiogenesis, cell motility, immune responses and stem cell differentiation (6-11).

Specificity/Sensitivity: Each phospho-Stat antibody in the kit recognizes only the phosphorylated form of its specific target.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptides corresponding to residues surrounding Tyr690 of human Stat2, Ser727 of mouse Stat3, or Tyr641 of human Stat6. Antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Tyr701 of human Stat1 protein, Tyr705 of mouse Stat3, or residues surrounding Tyr694 of human Stat5a protein.

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

Recommended Antibody Dilutions:

Western blotting	1:1000

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

Background References:

(1) Darnell Jr., J. et al. (1994) *Science* 264, 1415–1421.
(2) Leonard, W.J. and O'Shea, J.J. (1998) *Annu. Rev. Immunol.* 16, 293–322.
(3) Caldenhoven, E. et al. (1996) *J. Biol. Chem.* 271, 13221–13227.
(4) Wen, Z. et al. (1995) *Cell* 82, 241–250.
(5) Yokogami, K. et al. (2000) *Curr. Biol.* 10, 47–50.
(6) Lim, C.P. and Cao, X. (1999) *J. Biol. Chem.* 274, 31055–31061.
(7) Bromberg, J. F. et al. (1999) *Cell* 98, 295–303.
(8) Su, L. et al. (1999) *J. Biol. Chem.* 274, 31770–31774.
(9) Dentelli, P. et al. (1999) *J. Immunol.* 163, 2151–2159.
(10) Cattaneo, E. et al. (1999) *Trends Neurosci.* 22, 365–369.
(11) Frank, D.A. (1999) *Mol. Med.* 5, 432–456.

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

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.

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.