

#9350 Store at -20°C

PhosphoPlus® Stat5 (Tyr694) Antibody Kit



1 Kit
 (3 x 40 µl)

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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

| Products Included | Product # | Quantity | Mol. Wt. | Isotype |
|--|-----------|----------|----------|------------|
| Stat5 Antibody | 9363 | 40 µl | 90 kDa | Rabbit IgG |
| Phospho-Stat5 (Tyr694) Antibody | 9351 | 40 µl | 90 kDa | Rabbit IgG |
| Stat5 Control Cell Extracts | 9353 | 40 µl | | Rabbit IgG |
| Anti-rabbit IgG, HRP-linked Antibody | 7074 | 100 µl | | Goat |
| Anti-biotin, HRP-linked Antibody | 7075 | 100 µl | | Goat |
| Biotinylated Protein Ladder Detection Pack | 7727 | 100 µl | | |
| 20X LumiGLO® Reagent and 20X Peroxide | 7003 | 5 ml | | |

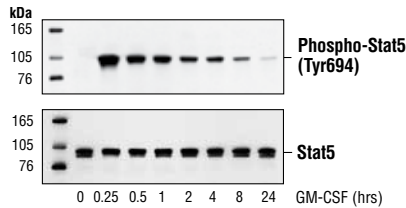
See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The PhosphoPlus® Stat5 (Tyr694) Antibody Kit provides reagents and protocols for the rapid analysis of the phosphorylation status of Stat5a at Tyr694 and Stat5b at Tyr699.

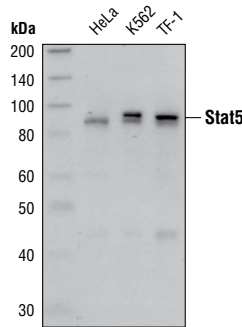
Background: Stat5 is activated in response to a wide variety of ligands including IL-2, GM-CSF, growth hormone and prolactin. Phosphorylation at Tyr694 is obligatory for Stat5 activation (1,2). This phosphorylation is mediated by Src upon erythropoietin stimulation (3). Stat5 is constitutively active in some leukemic cell types (4). Phosphorylated Stat5 is found in some endothelial cells treated with IL-3, which suggests its involvement in angiogenesis and cell motility (5). Stat5a and Stat5b are independently regulated and activated in various cell types. For instance, interferon treatment predominantly activates Stat5a in U-937 cells and Stat5b in HeLa cells (6).

Specificity/Sensitivity: Phospho-Stat5 (Tyr694) Antibody detects endogenous levels of Stat5a only when phosphorylated at Tyr694 and Stat5b when phosphorylated at Tyr699. This antibody does not cross-react with the corresponding phospho-tyrosine residues of other Stat proteins, but does cross-react with EGFR. Stat5 Antibody detects endogenous levels of total Stat5a and Stat5b.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues around Tyr694 of mouse Stat5a (Phospho-Stat5 (Tyr694) Antibody) or a synthetic peptide corresponding to an amino-terminal region of Stat5. Antibodies were purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from GM-CSF-treated (25 ng/ml) TF-1 cells, using **Phospho-Stat5 (Tyr694) Antibody #9351** (upper) or **Stat5 antibody #9352** (lower).



Western blot analysis of extracts from HeLa, K562 and TF-1 cells, using the **Stat5 Antibody #9363**.

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 a complete listing of recommended companion products.

Background References:

- (1) Gouilleux, F. et al. (1994) *EMBO J.* 13, 4361-4369.
- (2) Wakao, H. et al. (1994) *EMBO J.* 13, 2182-2191.
- (3) Okutani, Y. et al. (2001) *Oncogene* 20, 6643-6650.
- (4) Demoulin, J.B. et al. (1999) *J. Biol. Chem.* 274, 25855-25861.
- (5) Dentelli, P. et al. (1999) *J. Immunol.* 163, 2151-2159.
- (6) Meinke, A. et al. (1996) *Mol. Cell. Biol.* 16, 6937-6944.

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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 (Primary Antibody Incubation in BSA)

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.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope[®]-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO[®] chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO[®] (0.5 ml 20X LumiGLO[®], 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO[®] substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO[®] incubation and declines over the following 2 hours.