

**#9350** Store at -20°C

# PhosphoPlus® Stat5 (Tyr694) Antibody Kit



1 Kit  
 (10 western blots)

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

rev. 04/06/16

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Stat5 Antibody	9363	100 µl	90 kDa	Rabbit IgG
Phospho-Stat5 (Tyr694) (D47E7) XP® Rabbit mAb	4322	100 µl	90 kDa	Rabbit IgG
Stat5 Control Cell Extracts	9353	100 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	7727	100 µl		
20X LumiGLO® Reagent and 20X Peroxide	7003	5 ml		

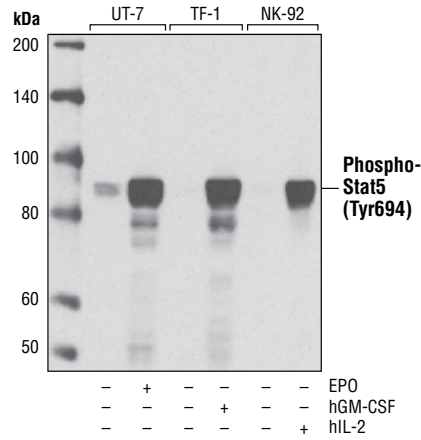
See [www.cellsignal.com](http://www.cellsignal.com) for individual component applications, species cross-reactivity, dilutions and additional application protocols.

**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) (D47E7) XPTM Rabbit mAb detects endogenous levels of Stat5a only when phosphorylated at Tyr694 and Stat5b when phosphorylated at Tyr699. Stat5 (3H7) Rabbit mAb detects endogenous levels of total Stat5α and β protein.

**Source/Purification:** Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Tyr694 of human Stat5a protein. Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to an amino-terminal region within Stat5. Antibodies were purified by protein A and peptide affinity chromatography.



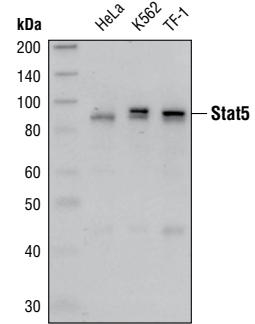
**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibodies.

**Recommended Antibody Dilutions:**  
 Western blotting 1:1000

**Please visit [www.cellsignal.com](http://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.



Western blot analysis of extracts from various cell lines using the Stat5 Antibody #9363.

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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.

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## 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.

- 20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH<sub>2</sub>O, mix.
- 10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH<sub>2</sub>O, mix.
- 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<sub>2</sub>O.
- 10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH<sub>2</sub>O, mix.
- 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<sub>2</sub>O, mix.
- 10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH<sub>2</sub>O, mix.
- Nonfat Dry Milk:** (#9999)
- 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.
- Wash Buffer:** (#9997) 1X TBST
- Bovine Serum Albumin (BSA):** (#9998)
- 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.
- Biotinylated Protein Ladder Detection Pack:** (#7727)
- Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
- Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
- Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
- Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

### B. Protein Blotting

**A general protocol for sample preparation.**

- 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 for a 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 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
- Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
- Microcentrifuge for 5 min.
- 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.
- Electrotransfer to nitrocellulose membrane (#12369).

### C. Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

#### I. Membrane Blocking

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

#### II. Primary Antibody Incubation

- 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.
- Wash three times for 5 min each with 15 ml of TBST.
- 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.
- Wash three times for 5 min each with 15 ml of TBST.
- Proceed with detection (Section D).

### D. Detection of Proteins

- 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.
- 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.

LumiGLO® is a registered trademark of Kirkegaard & Perry Laboratories. Tween® is a registered trademark of ICI Americas, INC. SignalFire™ is a trademark of Cell Signaling Technology, INC.

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# Material Safety Data Sheet (MSDS) for Antibodies

rev. 08/09/07

## I. Identification:

**Product name:** Antibodies

**Product Catalog Number:** Includes antibodies within the following range of catalog numbers: 2000-5999, 7000-7999 and 9000-9999.

**CAS number:** None

**Manufacturer Supplier:** Cell Signaling Technology  
3 Trask Lane  
Danvers, MA 01923 USA  
1-978-867-2300 TEL  
1-978-867-2400 FAX  
1-978-578-6737 Emergency Phone

## II. Composition/Information on Ingredients:

This product is composed of antibodies in aqueous buffer solution. According to 29 CFR 1910.1200(d), hazardous ingredients at less than <1% and carcinogens at less than <0.1% are considered non-hazardous. Any hazardous or carcinogenic ingredients exceeding these criteria are listed below.

This product may contain the following hazardous ingredients.

Ingredient	CAS#	Percent
Glycerol	56-81-5	50%

## III. Hazard Identification:

**Emergency Overview of Hazardous ingredient:** Glycerol (CAS# 56-81-5)

**Caution:** Avoid contact and inhalation.

**Target Organ:** Kidneys.

### NFPA Rating:

Health Rating:	1
Flammability Rating:	0
Reactivity Rating:	0

## IV. First Aid Measures:

**Inhalation:** If inhaled, remove to fresh air. If breathing is difficult, get medical attention.

**Ingestion:** If swallowed and person is conscious, rinse out mouth with water. Get medical attention.

**Skin Exposure:** In case of contact, wash skin with soap and water.

**Eye Exposure:** In case of contact with eyes, immediately flush eyes water for at least 15 minutes. Get medical attention.

## V. Fire Fighting Measures:

**Flash Point:** Data not available.

**Autoignition Temperature:** Data not available.

**Fire Extinguishing Media:** Water spray, dry chemical, foam, or carbon dioxide.

**Firefighting:** Wear protective clothing and self-contained breathing apparatus to prevent contact with skin and eyes.

## VI. Accidental Release Measures:

Absorb liquid with an absorbent material. Transfer contaminated absorbent to a chemical waste container for disposal.

## VII. Handling And Storage:

Avoid inhalation and contact with eyes and skin. Avoid prolonged or repeated exposure. Store at -20°C in tightly closed container.

## VIII. Exposure Controls/Personal

**Engineering Controls:** Maintain adequate ventilation, eye wash and quick-drench facilities in work area.

**Personal Protective Equipment:** Lab coat, chemical resistant gloves and chemical safety glasses.

**Occupational Exposure Limits:** Data not available.

## IX. Exposure Controls/Personal Protection:

<b>Physical State:</b>	Colorless liquid.
<b>Odor:</b>	Odorless.
<b>Boiling Point:</b>	Data not available.
<b>Melting Point:</b>	Data not available.
<b>Volatile Organic Compound:</b>	Data not available.
<b>Solubility in water:</b>	Readily miscible in water.

## X. Stability and Reactivity:

**Stability:** Stable.

**Hazardous Decomposition:** May form carbon dioxide and carbon monoxide.

**Conditions to avoid:** Strong oxidizing agents

## XI. Toxicological Information:

May cause skin irritation.

May be toxic if absorbed through skin or ingested.

May cause eye irritation.

**Target Organs:** Kidneys

Prolonged exposure may cause nausea, headache, and vomiting.

## XII. Ecological Information:

Data not available.

## XIII. Disposal Considerations:

Dispose of in accordance with federal, state and local environmental regulations.

## XIV. Transport Information:

**D.O.T.:** This substance is considered non-hazardous for transport.

**IATA:** This substance is considered non-hazardous for air transport.

## XV. Regulatory Information:

**EU Regulation/Classification/Labeling Information:** Not available for this product.

**Chemical Inventory Status:**

**SARA Listed Component:** None.

**TSCA Listed Component:** None.

**Canada (WHMIS):** DSL No, NDSL No.

## XVI. Other Information:

This compound is sold only for research use by personnel familiar with chemicals and who are well trained in good laboratory habits, such as avoiding spills, keeping hands clean at all times and not rubbing eyes with hands while working in the laboratory.

This solution is sold only in microliter quantities for use in life sciences research. No other use is intended, and any other use may involve substantive hazards.

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide for experienced personnel. Cell Signaling Technology, Inc., shall not be held liable for any damage resulting from the handling of or from contact with the above product. The burden of safe use of this material rests entirely with the user.