

# PhosphoPlus® Stat3 (Tyr705) Antibody Kit

- ✓ 1 Kit  
10 mini-blots  
(10 assays)

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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
Phospho-Stat3 (Tyr705) (D3A7) XP® Rabbit mAb	9145	100 µl	79, 86 kDa	Rabbit IgG
Stat3 Antibody	9132	100 µl	79, 86 kDa	Rabbit IgG
Stat3 Control Cell Extracts	9133	40 µl		
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.cellsignaling.com](http://www.cellsignaling.com) for individual component applications, species cross-reactivity, dilutions and additional application protocols.

**Background:** The Stat3 transcription factor is an important signaling molecule for many cytokines and growth-factor receptors (1) and is required for murine fetal development (2). Stat3 is constitutively activated in a number of human tumors (3,4) and possesses oncogenic potential (5) and anti-apoptotic activities (3). Stat3 is activated by phosphorylation at Tyr705, which induces dimerization, nuclear translocation and DNA binding (6,7). Transcriptional activation seems to be regulated by phosphorylation at Ser727 through the MAPK or mTOR pathways (8,9). Stat3 isoform expression appears to reflect biological function as the relative expression levels of Stat3α (86 kDa) and Stat3β (79 kDa) depend on cell type, ligand exposure or cell maturation stage (10). It is notable that Stat3β lacks the serine phosphorylation site within the carboxy-terminal transcriptional activation domain (8).

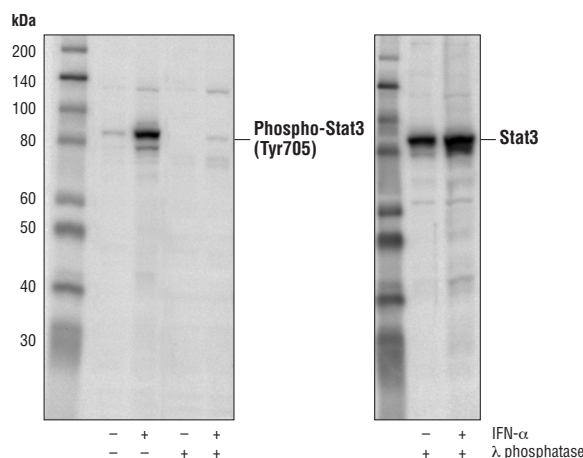
**Specificity/Sensitivity:** Phospho-Stat3 (Tyr705) Antibody detects endogenous levels of Stat3 only when phosphorylated at tyrosine 705. The antibody does not cross-react with other Stat proteins when phosphorylated on the corresponding tyrosine residue, but does cross-react with EGFR. Stat3 antibody detects endogenous levels of total Stat3 protein.

**Source/Purification:** PMonoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Tyr705 of mouse Stat3. Polyclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to the sequence of mouse Stat3 and purified by protein A and peptide affinity chromatography.

Western blot analysis of extracts from IFN-α stimulated HeLa cells alone or with λ phosphatase treatment using **Phospho-Stat3 (Tyr705) (D3A7) XP® Rabbit mAb #9145 (left)** or **Stat3 Antibody #9132 (right)**.

## Background References:

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- Catlett-Falcone, R. et al. (1999) *Immunity* 10, 105-115.
- Garcia, R. and Jove, R. (1998) *J. Biomed. Sci.* 5, 79-85.
- Bromberg, J.F. et al. (1999) *Cell* 98, 295-303.
- Darnell Jr., J.E. et al. (1994) *Science* 264, 1415-1421.
- Ihle, J.N. (1995) *Nature* 377, 591-594.
- Wen, Z. et al. (1995) *Cell* 82, 241-250.
- Yokogami, K. et al. (2000) *Curr. Biol.* 10, 47-50.
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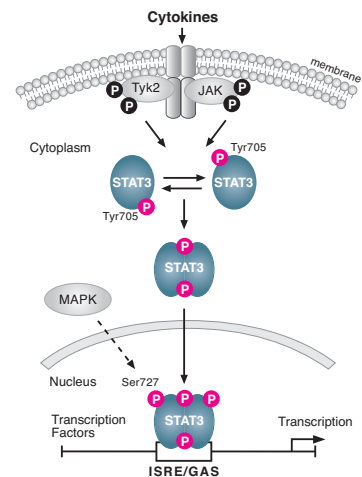


**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibodies.

## Recommended Antibody Dilutions:

Western blotting 1:1000

Please visit [www.cellsignaling.com](http://www.cellsignaling.com) for a complete listing of recommended companion products.



## 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<sup>®</sup>-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<sup>®</sup> 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 cm<sup>2</sup>) 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<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO<sup>®</sup> 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<sup>®</sup> incubation and declines over the following 2 hours.