#9364 Store at –20°C

Phospho-Stat6 (Tyr641) (C11A12) Rabbit mAb

☐ Small 100 µl
(10 Western mini-blots)

☐ Petite 40 µl
(4 Western mini-blots)

Cell Signaling TECHNOLOGY®

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New 04/07

Applications	Species Cross-Reactivity*	Molecular Wt.	Source	Isotype
W	H, (Mk)	100 kDa	Rabbit**	IgG

Background: Upon activation by Janus kinases, Stat6 translocates to the nucleus where it regulates cytokine-induced gene expression. Stat6 is activated via phosphorylation at Tyr641 and is required for responsiveness to IL-4 and IL-13 (1–4). In addition, Stat6 is activated by IFN-α in B cell lines, where it forms transcriptionally active complexes with Stat2 and p48 (5,6). Protein phosphatase 2A is also involved in regulation of IL-4-mediated Stat6 signaling (7).

Specificity/Sensitivity: Phospho-Stat6 (Tyr641) (C11A12) Rabbit mAb detects endogenous levels of Stat6 only when phosphorylated at Tyr641. This antibody does not cross-react with the corresponding phospho-tyrosine residues of other Stat proteins. Cross-reactivity has been observed with receptor tyrosine kinases including phosphorylated PDGF receptor that can lead to inapproriate membrane staining.

Source/Purification: Rabbit monoclonal antibodies were prepared from spleens of rabbits immunized with a synthetic phosphopeptide (KLH-coupled) corresponding to residues surrounding Tyr641 of human Stat6.

Background References:

(1) Nelms, K. et al. (1999) *Ann. Rev. Immunol.* 17, 701–738.

(2) Malabarba, M.G. et al. (1996) *Biochem. J.* 319, 865–872.

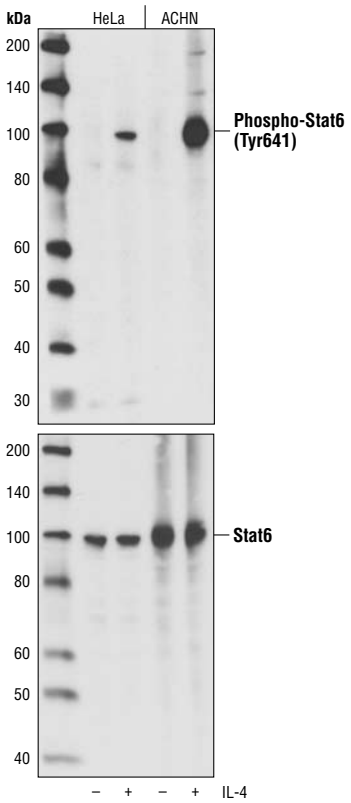
(3) Hou, J. et al. (1994) *Science* 265, 1701–1706.

(4) Quelle, F.W. et al. (1995) *Mol. Cell. Biol.* 15, 3336–3343.

(5) Takeda, K. et al. (1996) *Nature* 380, 627–630.

(6) Gupta, S. et al. (1999) *J. Immunol.* 163, 3834–3841.

(7) Woetmann, A. et al. (2003) *J. Biol. Chem.* 278, 2787–2791.



Western blot analysis of extracts from HeLa and ACHN cells, untreated or treated with IL-4 (15 min), using Phospho-Stat6 (Tyr641) (C11A12) Rabbit mAb (upper) or Stat6 Antibody #9362 (lower).

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 antibody.

***Species cross-reactivity is determined by Western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:
Western blotting 1:1000

Companion Products:

Phospho-Stat6 (Tyr641) Antibody #9361

Stat6 Antibody #9362

Phospho-Stat1 (Tyr701) (58D6) Rabbit mAb #9167

Phospho-Stat2 (Tyr690) Antibody #4441

Phospho-Stat3 (Tyr705) (D3A7) Rabbit mAb #9145

Phospho-Stat5 (Tyr694) (C11C5) Rabbit mAb #9359

Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071

Anti-rabbit IgG, HRP-linked Antibody #7074

Prestained Protein Marker, Broad Range (Premixed Format) #7720

Biotinylated Protein Ladder Detection Pack #7727

20X LumiGLO® Reagent and 20X Peroxide #7003

IMPORTANT: 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.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **IC**—Immunocytochemistry **IF**—Immunofluorescence **F**—Flow cytometry **E**—ELISA **D**—DELFIA®

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **X**—Xenopus **Z**—zebra fish **B**—bovine **All**—all species expected

Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Western Immunoblotting Protocol (Primary Ab 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.

1. 1X Phosphate Buffered Saline (PBS)
2. **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
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **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).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **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%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **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%).
10. **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.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **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.

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 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.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. 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.

8. Electrotransfer to nitrocellulose or PVDF membrane.

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.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. 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.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. 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.

2. 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.