

#9153 Store at -20°C

# LATS1 Antibody

✓ 100 µl  
(10 western blots)

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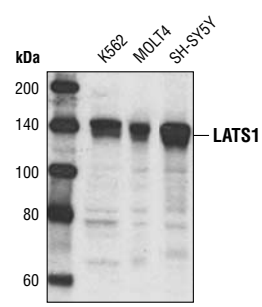
This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W Endogenous	H, Mk	140 kDa	Rabbit**

**Background:** LATS1 (Large tumor suppressor 1) is putative serine/threonine kinase that belongs to the NDR family (1). It is a tumor suppressor that plays a critical role in maintenance of ploidy. LATS1 localizes to the centrosome and the mitotic spindle and controls G(2)/M transition by negatively regulating CDC2 kinase activity (2,3). It also plays a role in the G1 tetraploidy checkpoint via control of p53 expression (4). LATS1 affects cytokinesis by regulating actin polymerization through negative modulation of LIMK1 (5). LATS1 also binds the phosphorylated form of zyxin, a regulator of actin filament assembly. This interaction promotes localization of zyxin to the mitotic spindle, suggesting a role for actin regulatory proteins during mitosis (6). Decreased expression is associated with breast tumor aggressiveness (7), and promoter methylation, loss of heterozygosity, and mutations perturbing LATS1 have been associated with human sarcomas and ovarian sarcomas (8,9). LATS1 knock out mice develop soft-tissue sarcomas, ovarian stromal cell tumors and display a high sensitivity to carcinogenic treatments (10). It has recently been shown that human LATS1 exists in a complex similar to the Drosophila Hippo/Salvador/Lats tumor suppressor network, a complex that regulates proliferation and apoptosis to control growth and shape of the fly. The human complex contains Hippo and Salvador homologs RASSF1A, WW45, and also MST2, and may control mitotic exit (11).

**Specificity/Sensitivity:** LATS1 Antibody detects endogenous levels of total LATS1 protein.

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to amino acids surrounding Ser177 of human LATS1. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis from extracts of K562, MOLT4 and SH-SY5Y cells using LATS1 Antibody.

### Background References:

- (1) Tao, W. et al. (1999) *Nat Genet* 21, 177–81.
- (2) Yang, X. et al. (2001) *Oncogene* 20, 6516–23.
- (3) Xia, H. et al. (2002) *Oncogene* 21, 1233–41.
- (4) Iida, S. et al. (2004) *Oncogene* 23, 5266–74.
- (5) Yang, X. et al. (2004) *Nat Cell Biol* 6, 609–17.
- (6) Hirota, T. et al. (2000) *J Cell Biol* 149, 1073–86.
- (7) Morinaga, N. et al. (2000) *Int J Oncol* 17, 1125–9.
- (8) Hansen, L.L. et al. (2002) *Cancer Genet Cytogenet* 139, 1–8.
- (9) Hisaoka, M. et al. (2002) *Lab Invest* 82, 1427–35.
- (10) St John, M.A. et al. (1999) *Nat Genet* 21, 182–6.
- (11) Guo, C. et al. (2007) *Curr Biol* 17, 700–5.

Entrez-Gene ID # 9113  
Swiss-Prot Acc. # O95835

**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.

\*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

For application specific protocols please see the web page for this product at [www.cellsignal.com](http://www.cellsignal.com).

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

**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 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.

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<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.
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<sup>2</sup>) 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<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.

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<sup>®</sup> incubation and declines over the following 2 hours.