

Phospho-LKB1 (Ser428) Antibody

100 µl
 (10 Western mini-blot)

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This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W Transfected	H	54 kDa	Rabbit

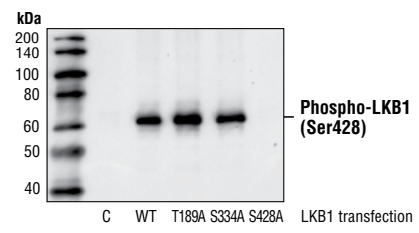
Background: Peutz-Jeghers syndrome (PJS) is caused by mutation of the tumor suppressor kinase, LKB1 (1,2). The amino-terminal noncatalytic domain of LKB1 comprises both a nuclear localization signal (3) and a putative cytoplasmic retention signal (4). LKB1 is phosphorylated in cells at Ser31, 325 and 431, and at Thr189, 336 and 366 (5,6). LKB1 activates AMP-activated protein kinase (AMPK) via phosphorylation at Thr172 (7-9).

AMPK plays a key role in the regulation of energy homeostasis, sensing conditions of low ATP and signaling to downstream effector molecules that conserve ATP and regulate cell growth and apoptosis.

Specificity/Sensitivity: Phospho-LKB1 (Ser428) Antibody detects transfected levels of LKB1 only when phosphorylated at serine 428. The antibody does not cross-react with LKB1 phosphorylated at other sites.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Ser428 of human LKB1. Antibodies are purified by protein A and peptide affinity chromatography.

- Background References:**
- (1) Hemminki, A. et al. (1998) *Nature* 391, 184-187.
 - (2) Jenne, D.E. et al. (1998) *Nat. Genet.* 18, 38-43.
 - (3) Smith, D.P. et al. (1999) *Hum. Mol. Genet.* 8, 1479-1485.
 - (4) Nezu, J. et al. (1999) *Biochem. Biophys. Res. Commun.* 261, 750-755.
 - (5) Sapkota, G.P. et al. (2001) *J. Biol. Chem.* 276, 19469-19482.
 - (6) Sapkota, G.P. et al. (2002) *Biochem. J.* 362, 481-490.
 - (7) Hawley, S.A. et al. (2003) *J. Biol.* 2, 28.
 - (8) Woods, A. et al. (2003) *Curr. Biol.* 13, 2004-2008.
 - (9) Shaw, R.J. et al. (2004) *Proc. Natl. Acad. Sci. USA* 101, 3329-3335.



Western blot analysis of extracts from COS cells, untransfected or transfected with Wild-type LKB1, LKB1 (T189A), LKB1 (S334A) or LKB1 (S428A), using Phospho-LKB1 (Ser428) Antibody.

Entrez-Gene ID # 6794
Swiss-Prot Acc. # Q15831c

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.

Recommended Antibody Dilutions:
 Western blotting 1:1000

- Companion Products:**
- Phospho-LKB1 (Thr189) Antibody #3054
 - Phospho-LKB1 (Ser334) Antibody #3055
 - Phospho-AMPKα (Thr172) Antibody #2531
 - 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.

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.