

#3674 Store at -20°C

# Phospho-Myosin Light Chain 2 (Thr18/Ser19) 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.

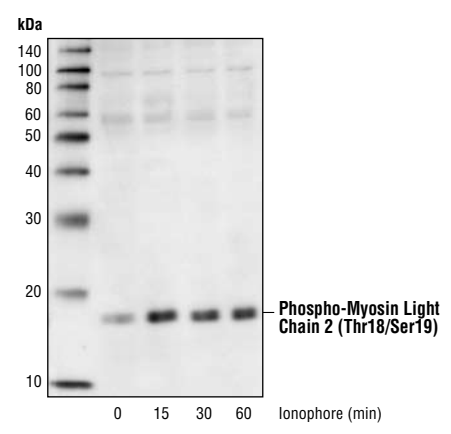
Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W	H, M, B, (R, C, X, Pg)	18 kDa	Rabbit

**Background:** Myosin is composed of six polypeptide chains: two identical heavy chains and two pairs of light chains. Myosin light chain 2 (MLC2), also known as myosin regulatory light chain (MRLC or RLC, LC20), has many isoforms depending on its distribution. In smooth muscle, MLC2 is phosphorylated at Thr18 and Ser19 by myosin light chain kinase (MLCK) in a Ca<sup>2+</sup>/calmodulin-dependent manner (1). This phosphorylation is correlated with myosin ATPase activity and smooth muscle contraction (2). ROCK also phosphorylates Ser19 of smooth muscle MLC2, which regulates the assembly of stress fibers (3). Phosphorylation of smooth muscle MLC2 at Ser1/Ser2 and Ser9 by PKC and cdc2 has been reported to inhibit myosin ATPase activity (4,5). Phosphorylation by cdc2 controls the timing of cytokinesis (5). Transgenic mice lacking phosphorylation sites on the cardiac muscle isoform show morphological and functional abnormalities (6).

**Specificity/Sensitivity:** Phospho-Myosin Light Chain 2 (Thr18/Ser19) Antibody detects endogenous levels of myosin light chain 2 (smooth muscle) only when dually phosphorylated at Thr18 and Ser19. The antibody does not cross-react with the cardiac isoform of myosin light chain 2.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Thr18/Ser19 of human myosin light chain 2. Antibodies are purified by protein A and peptide affinity chromatography.

**Selected Application References:**  
Birukova, A.A. et al. (2004) Microtubule Disassembly Induces Cytoskeletal Remodeling and Lung Vascular Barrier Dysfunction: Role of Rho-Dependent Mechanisms. *J. Cellular Phys.* 201, 55–50. Applications: W, IC-IF.



Western blot analysis of extracts from HEK293 cells stimulated with ionophore A23187 for the indicated times using Phospho-Myosin Light Chain 2 (Thr18/Ser19) Antibody.

**Background References:**

- (1) Ikebe, M. and Hartshorne, D.J. (1985) *J. Biol. Chem.* 260, 10027–10031.
- (2) Tan, J. L. et al. (1992) *Annu. Rev. Biochem.* 61, 721–759.
- (3) Totsukawa, G. et al. (2000) *J. Cell Biol.* 150, 797–806.
- (4) Ikebe, M. et al. (2000) *J. Biol. Chem.* 262, 9569–9573.
- (5) Satterwhite, L.L. et al. (1992) *J. Cell Biol.* 118, 595–605.
- (6) Sanbe, A. et al. (1999) *J. Biol. Chem.* 274, 21085–21094.

**Entrez-Gene ID** #10398  
**Swiss-Prot Acc.** #P24844

**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-Myosin Light Chain 2 (Ser19) Antibody #3671  
Myosin Light Chain 2 Antibody #3672  
Phospho-Myosin Light Chain 2 (Ser19) Mouse mAb #3675  
Anti-rabbit IgG, HRP-linked Antibody #7074  
Prestained Protein Marker, Broad Range (Premixed Format) #7720  
Biotinylated Protein Ladder #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—DELFIATM  
**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 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 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® (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.