

# Myosin Light Chain 2 Antibody Sampler Kit



✓ 1 Kit  
(3 x 20 µl)

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**For Research Use Only. Not For Use In Diagnostic Procedures.**

| Products Included                                   | Product # | Quantity | Mol. Wt. | Isotype    |
|---|-----------|----------|----------|------------|
| Myosin Light Chain 2 (D18E2) Rabbit mAb             | 8505      | 20 µl    | 18 kDa   | Rabbit IgG |
| Phospho-Myosin Light Chain 2 (Ser19) Antibody       | 3671      | 20 µl    | 18 kDa   | Rabbit IgG |
| Phospho-Myosin Light Chain 2 (Thr18/Ser19) Antibody | 3674      | 20 µl    | 18 kDa   | Rabbit IgG |
| Anti-rabbit IgG, HRP-linked Antibody                | 7074      | 100 µl   |          | Goat       |

See [www.cellsignal.com](http://www.cellsignal.com) for individual component applications, species cross-reactivity, dilutions and additional application protocols.

**Description:** The Myosin Light Chain 2 Antibody Sampler Kit provides an economical means to detect total, phosphorylated, and dual-phosphorylated myosin light chain 2. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

**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:** The antibodies in the Myosin Light Chain 2 Antibody Sampler Kit detect endogenous levels of total myosin light chain 2 (smooth muscle), myosin light chain 2 when phosphorylated at Ser19 or when dually phosphorylated at Thr18 and Ser19, respectively. The antibodies do not cross-react with the cardiac isoform of Myosin Light Chain 2.

**Source/Purification:** The rabbit monoclonal antibody is produced by immunizing rabbits with a synthetic peptide corresponding to residues near the carboxy terminus of human myosin light chain 2 protein. Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser19 of human myosin light chain 2, or to residues surrounding Thr18/Ser19 of human myosin light chain 2, respectively.

**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 antibodies.

**Recommended Antibody Dilutions:**  
Western blotting 1:1000

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

Polyclonal antibodies are purified by protein A and peptide affinity chromatography.

**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.

## Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

**NOTE:** Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

### A. Solutions and Reagents

**NOTE:** Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

- 20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH<sub>2</sub>O, mix.
- 10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH<sub>2</sub>O, mix.
- 1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723)  
Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH<sub>2</sub>O.
- 10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH<sub>2</sub>O, mix.
- 10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH<sub>2</sub>O, mix.
- 10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH<sub>2</sub>O, mix.
- Nonfat Dry Milk:** (#9999)
- Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
- Wash Buffer:** (#9997) 1X TBST
- Bovine Serum Albumin (BSA):** (#9998)
- Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
- Biotinylated Protein Ladder Detection Pack:** (#7727)
- Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
- Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
- Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
- Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

### B. Protein Blotting

**A general protocol for sample preparation.**

- 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 for a 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 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
- Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
- Microcentrifuge for 5 min.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
- Electrotransfer to nitrocellulose membrane (#12369).

### 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.

#### I. Membrane Blocking

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
- Wash three times for 5 min each with 15 ml of TBST.

#### II. Primary Antibody Incubation

- Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 min each with 15 ml of TBST.
- Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
- Wash three times for 5 min each with 15 ml of TBST.
- Proceed with detection (Section D).

### D. Detection of Proteins

- Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time.  
**NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.

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