

Myosin II Isoform Antibody Sampler Kit

✓ 1 Kit
(3 x 40 µl)

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This product is for *in vitro* research use only and is not intended for use in humans or animals.
This product is not intended for use as a therapeutic or in diagnostic procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Myosin IIa Antibody	3403	40 µl	230 kDa	Rabbit IgG
Myosin IIb Antibody	3404	40 µl	230 kDa	Rabbit IgG
Myosin IIc Antibody	3405	40 µl	230 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The Myosin II Isoform Antibody Sampler Kit is an economical way to examine the total protein levels of myosin II isoforms a, b, and c. The kit includes enough primary and secondary antibodies to perform four western mini-blot experiments.

Background: Nonmuscle myosin is an actin-based motor protein essential to cell motility, cell division, migration, adhesion and polarity. The holoenzyme consists of two identical heavy chains and two sets of light chains. The light chains (MLCs) regulate myosin II activity and stability. The heavy chains (NMHCs) are encoded by three genes, MYH9, MYH10 and MYH14, which generate three different nonmuscle myosin II isoforms, IIa, IIb and IIc, respectively (reviewed in 1). While all three isoforms perform the same enzymatic tasks, namely binding to and contracting actin filaments coupled to ATP hydrolysis, their cellular functions do not appear to be redundant and they have different subcellular distributions (2-5). The carboxy-terminal tail domain of myosin II is important in isoform-specific subcellular localization (6).

Specificity/Sensitivity: Each antibody in the Myosin II Isoform Antibody Sampler Kit detects endogenous total levels of the specified myosin II isoform.

Source/Purification: Polyclonal antibodies are produced by immunizing animals with synthetic peptides (KLH-coupled) corresponding to the carboxy terminus of mouse myosin IIa and human myosin IIb and IIc.

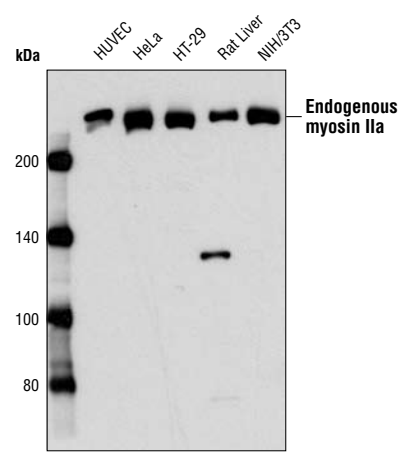
Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibodies.

Recommended Antibody Dilutions:
Western blotting 1:1000

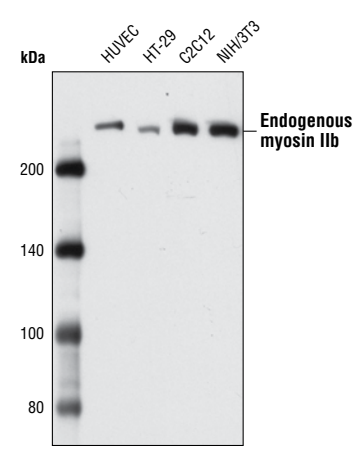
Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

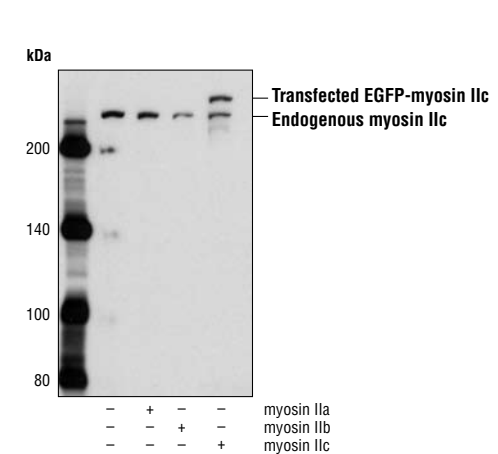
- (1) Conti, M.A. and Adelstein, R.S. (2008) *J Cell Sci* 121, 11–18.
- (2) Sandquist, J.C. et al. (2006) *J Biol Chem* 281, 35873–83.
- (3) Even-Ram, S. et al. (2007) *Nat Cell Biol* 9, 299–309.
- (4) Vicente-Manzanares, M. et al. (2007) *J Cell Biol* 176, 573–80.
- (5) Wylie, S.R. and Chantler, P.D. (2008) *Mol Biol Cell* 19, 3956–68.
- (6) Sandquist, J.C. and Means, A.R. (2008) *Mol Biol Cell* 19, 5156–67.



Western blot analysis of extracts from various cell types using **Myosin IIa Antibody #3403**.



Western blot analysis of extracts from various cell types using **Myosin IIb Antibody #3404**.



Western blot analysis of extracts from from COS cells, untransfected (mock) or transfected with nonmuscle EGFP-myosin IIa, IIb or IIc, using **Myosin IIc Antibody #3405**.

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 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.

- 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.