Store at -20°C

#5143

MYPT1 Antibody Sampler Kit

Cell Signaling TECHNOLOGY®

✓ 1 Kit
(5 x 40 µl)

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

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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
Phospho-MYPT1 (Ser507) Antibody	3040	40 µl	140 kDa	Rabbit IgG
Phospho-MYPT1 (Ser668) Antibody	3048	40 µl	140 kDa	Rabbit IgG
Phospho-MYPT1 (Thr696) Antibody	5163	40 µl	140 kDa	Rabbit IgG
Phospho-MYPT1 (Thr853) Antibody	4563	40 µl	140 kDa	Rabbit IgG
MYPT1 Antibody	2634	40 µl	140 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.

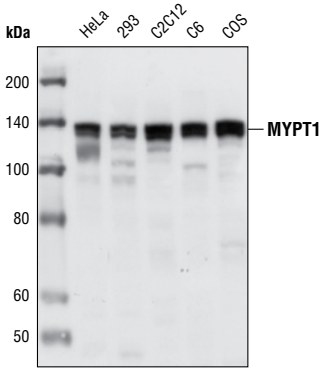
Description: The MYPT1 Antibody Sampler Kit is an economical way to examine the total protein levels of MYPT1 as well as MYPT1 phosphorylated at Ser507, Ser668, and Thr853. The kit includes enough primary and secondary antibodies to perform four western mini-blot experiments.

Background: Protein phosphatase 1 (PP1) is a ubiquitous eukaryotic protein serine/threonine phosphatase involved in the regulation of various cell functions. Substrate specificity is determined by the binding of a regulatory subunit to the PP1 catalytic subunit (PP1c). It is estimated that over fifty different regulatory subunits exist (1).

The myosin phosphatase holoenzyme is composed of three subunits: the PP1 catalytic subunit (PP1c), a targeting/regulatory subunit (MYPT/myosin-binding subunit of myosin phosphatase) and a 20 kDa subunit of unknown function (M20). MYPT binding to PP1cδ alters the conformation of the catalytic cleft and increases enzyme activity and specificity (2). Two MYPT isoforms that are 61% identical have been described. MYPT1 is widely expressed while MYPT2 expression appears to be exclusive to heart and brain (3). Related family members also include MBS85, MYPT3 and TIMAP (4).

Myosin phosphatase regulates the interaction of actin and myosin in response to signaling through the small GTPase Rho. Rho activity inhibits myosin phosphatase via Rho-associated kinase (ROCK). Phosphorylation of MYPT1 at Thr696 and Thr853 results in phosphatase inhibition and cytoskeletal reorganization (5,6).

Phospho-MYPT1 (Ser507) and phospho-MYPT1 (Ser668) antibodies are directed at sites that were identified at Cell Signaling Technology (CST) using PhosphoScan®, CST's LC-MS/MS platform for modification site discovery. Phosphorylation at these sites was discovered using an Akt substrate antibody. Please visit PhosphoSitePlus™, CST's modification site knowledgebase, at www.phosphosite.org for more information.



*Western blot analysis of extracts from various cell types using **MYPT1 Antibody #2634**.*

Specificity/Sensitivity: Each antibody in the MYPT1 Antibody Sampler Kit detects endogenous levels of its target protein. Activation state antibodies detect only target proteins phosphorylated at indicated residues. The MYPT1 Antibody is not likely to cross-react with MYPT2 nor does it cross-react with other family members.

Source/Purification: Phospho-specific polyclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding Ser507, Ser668, Thr853, and Thr696 of human MYPT1. MYPT1 Antibody is produced by immunizing animals with a synthetic peptide corresponding to amino-terminal residues of human MYPT1. Antibodies are purified using protein A and peptide affinity chromatography.

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) Cohen, P.T. (2002) *J Cell Sci* 115, 241–56.
(2) Terrak, M. et al. (2004) *Nature* 429, 780–4.
(3) Fujioka, M. et al. (1998) *Genomics* 49, 59–68.
(4) Ito, M. et al. (2004) *Mol Cell Biochem* 259, 197–209.
(5) Birukova, A.A. et al. (2004) *Microvasc Res* 67, 64–77.
(6) Birukova, A.A. et al. (2004) *J Cell Physiol* 201, 55–70.

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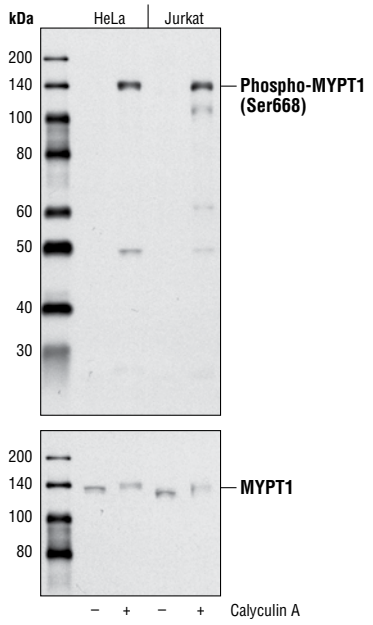
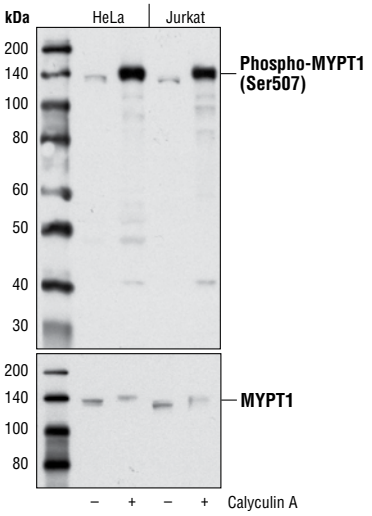
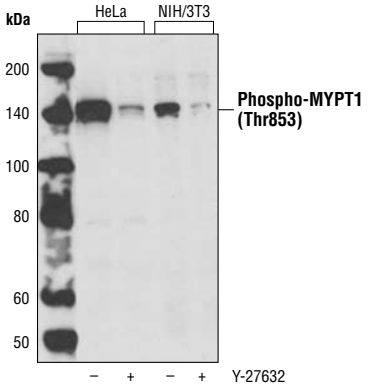
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **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.



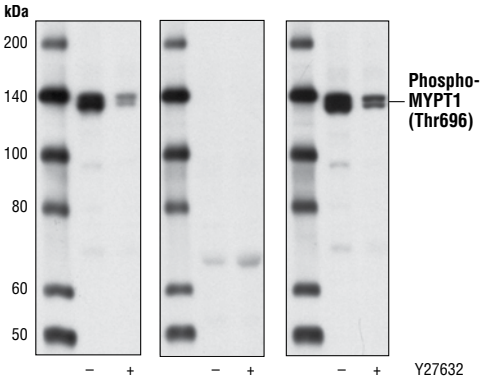
Western blot analysis of extracts from HeLa and Jurkat cells, untreated or treated with Calyculin A #9902, using ***Phospho-MYPT1 (Ser668) Antibody #3048*** *(upper) or MYPT1 Antibody #2634 (lower).*

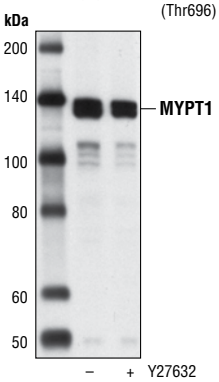


Western blot analysis of extracts from HeLa and Jurkat cells, untreated or treated with Calyculin A #9902, using ***Phospho-MYPT1 (Ser507) Antibody #3040*** *(upper) or MYPT1 Antibody #2634 (lower).*



Western blot analysis of extracts from HeLa or NIH/3T3 cells, untreated or treated with the ROCK inhibitor Y-27632, using ***Phospho-MYPT1 (Thr853) Antibody #4563.***





Western blot analysis of extracts from 293 cells, untreated or treated with the ROCK inhibitor Y27632 (2 minutes at 10 μM) using ***Phospho-MYPT1 (Thr696) Antibody #5163*** *or total MYPT1 Antibody #2634. Phospho-MYPT1 (Thr696) Antibody was pre-incubated as indicated with phospho-MYPT1 (Thr696) peptide or nonphospho-MYPT1 (Thr696) peptide.*

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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®-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.
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^2) 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® (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.

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® incubation and declines over the following 2 hours.

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