

#3251 Store at -20°C

Phospho-Caveolin-1 (Tyr14) Antibody

✓ 100 µl (10 Western mini-blots)

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

rev. 10/16/07

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

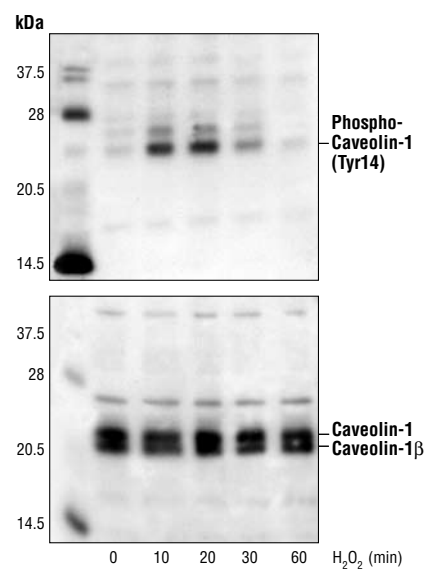
Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W	H, M, R, Mk	23, 25 kDa	Rabbit

Background: The 21-24 kDa integral proteins, caveolins, are the principal structural components of the cholesterol/sphingolipid-enriched plasma membrane microdomain caveolae. Three members of the caveolins family (caveolin-1, -2 and -3) have been identified with different tissue distributions. Caveolins form hetero- and homooligomers, which interact with cholesterol and other lipids (1). Caveolins are involved in diverse biological functions, including vesicular trafficking, cholesterol homeostasis, cell adhesion and apoptosis, and are also implicated in neurodegenerative disease (2). Caveolins also interact with multiple signaling molecules, such as G-α subunit, tyrosine kinase receptors, PKCs, Src family tyrosine kinases and eNOS (1,2). It is believed that caveolins serve as scaffolding proteins for the integration of signal transduction. Phosphorylation at Tyr14 is essential for caveolin association with SH2 or PTB domain-containing adaptor proteins, such as GRB7 (3-5). Phosphorylation at Ser80 regulates caveolin binding to the ER membrane and entry into the secretory pathway (6).

Specificity/Sensitivity: Phospho-Caveolin-1 (Tyr14) Antibody detects endogenous levels of caveolin-1 only when phosphorylated at Tyr14. The antibody does not cross-react with caveolin-2, -3 or caveolin-1β, the short isoform of caveolin-1.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic phospho-peptide (KLH-coupled) corresponding to residues surrounding Tyr14 of human caveolin-1. Antibodies are purified by protein A and peptide affinity chromatography.

Selected Application References:
Salatino, M. et al. (2006) Progesterin-induced caveolin-1 expression mediates breast cancer cell proliferation. *Oncogene* 25, 7723-39. Application: W.



Western blotting analysis of extracts from HeLa cells treated with H₂O₂ for the indicated times using Phospho-Caveolin-1 (Tyr14) Antibody (upper) or caveolin-1 antibody (lower).

Background References:

- (1) Okamoto, T. et al. (1998) *J. Biol. Chem.* 273, 5419-5422.
- (2) Smart, E.J. et al. (1999) *Mol. Cell. Biol.* 19, 7289-7304.
- (3) Nomura, R. et al. (1999) *Mol. Biol. Cell* 10, 975-986.
- (4) Volonte, D. et al. (2001) *J. Biol. Chem.* 276, 8094-8103.
- (5) Lee, H. et al. (2000) *Mol. Endocrinol.* 14, 1750-1775.
- (6) Schlegel, A. et al. (2001) *J. Biol. Chem.* 276, 4398-4408.

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:
Caveolin-1 Antibody #3238
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 #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.