

#3878 Store at -20°C

Phospho-Vimentin (Ser82) Antibody

- Small 100 µl (10 Western mini-blot)
- Petite 40 µl (4 Western mini-blot)



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New 07/07

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

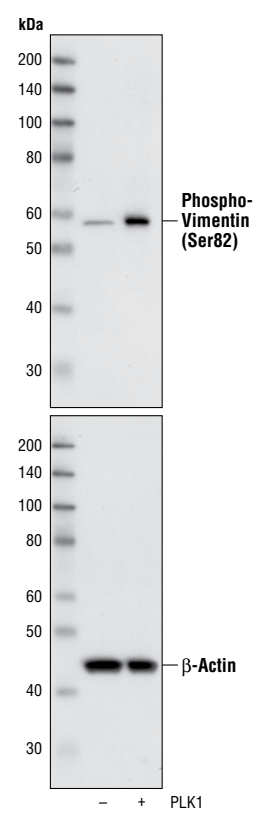
Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W	H, M, R, Mk	57 kDa	Rabbit

Background: The cytoskeleton consists of three types of cytosolic fibers: microfilaments (actin filaments), intermediate filaments and microtubules. Major types of intermediate filaments are distinguished and expressed in particular cell types: cytokeratins (epithelial cells), glial fibrillary acidic protein, GFAP (glial cells), desmin (skeletal, visceral and certain vascular smooth muscle cells), vimentin (mesenchyme origin) and neurofilaments (neurons). GFAP and vimentin form intermediate filaments in astroglial cells and modulate their motility and shape (1). In particular, vimentin filaments are present at early developmental stages, while GFAP filaments are characteristic of differentiated and mature brain astrocytes. Thus, GFAP is commonly used as a marker for intracranial and intraspinal tumors arising from astrocytes (2). Vimentin is present in sarcomas, but not carcinomas, and its expression is examined in conjunction with that of other markers to distinguish between the two (3). Vimentin's dynamic structural changes and spatial re-organization in response to extracellular stimuli helps to coordinate various signaling pathways (4). Phosphorylation of vimentin at Ser56 in smooth muscle cells regulates structural arrangement of vimentin filaments in response to serotonin (5,6). Remodeling of vimentin and other intermediate filaments is important during lymphocyte adhesion and migration through the endothelium (7).

During mitosis, CDK1 phosphorylates vimentin at Ser56. This phosphorylation provides a PLK binding site for vimentin-PLK interaction. PLK further phosphorylates vimentin at Ser82, which might serve as memory phosphorylation site and play a regulatory role in vimentin filament disassembly (8,9).

Specificity/Sensitivity: Phospho-Vimentin (Ser82) Antibody detects endogenous levels of vimentin only when phosphorylated at Ser82.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic phosphopeptide (KLH-coupled) corresponding to residues surrounding Ser82 of human vimentin. Antibodies are purified by peptide affinity chromatography.



Western blot analysis of extracts from HeLa cells, untreated or phosphorylated *in vitro* by PLK, using Phospho-Vimentin (Ser82) Antibody (upper). β-Actin Antibody #4967 (lower) was used as a loading control.

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-Vimentin (Ser56) Antibody #3877
 - β-Actin Antibody #4967
 - Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
 - Anti-rabbit IgG, HRP-linked Antibody #7074
 - Anti-biotin, HRP-linked Antibody #7075
 - Prestained Protein Marker, Broad Range (Premixed Format) #7720
 - Biotinylated Protein Ladder Detection Pack #7727
 - 20X LumiGLO® Reagent and 20X Peroxide #7003

- Background References:**
- (1) Eng, L.F. et al. (2000) *Neurochem. Res.* 25, 1439–1451.
 - (2) Goebel, H.H. et al. (1987) *Acta Histochem. Suppl.* 34, 81–93.
 - (3) Leader, M. et al. (1987) *Histopathology* 11, 63–72.
 - (4) Helfand, B.T. et al. (2004) *J. Cell Sci.* 117, 133–141.
 - (5) Tang, D.D. et al. (2005) *Biochem. J.* 388, 773–783.
 - (6) Fomina, I.G. et al. (1990) *Klin. Med. (Mosk.)* 68, 125–127.
 - (7) Nieminen, M. et al. (2006) *Nat. Cell Biol.* 8, 156–162.
 - (8) Yamaguchi, T. et al. (2005) *J Cell Biol* 171, 431–436.
 - (9) Oguri, T. et al. (2006) *Genes Cells* 11, 531–540.

IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

Western Immunoblotting Protocol (Primary Ab Incubation In nonfat dry milk)

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% nonfat dry milk; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk 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.