

#2800 Store at -20°C

# Claspin Antibody

✓ 100 µl (10 Western mini-blot)

Orders ■ 877-616-CELL (2355) orders@cellsignal.com  
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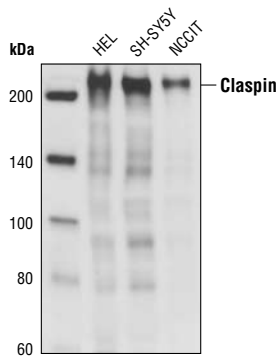
This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W	H	220 kDa	Rabbit

**Background:** Originally identified in *Xenopus* (1), and later in human cells (2), claspin is a mediator of Chk1 signal transduction at the replication checkpoint and in response to DNA damage. Expression of claspin is cell cycle-regulated, with protein levels peaking at the S/G2 phase (2). Expression is negatively regulated by both proteasome- and caspase-mediated degradation (3), and stabilized by activation of Chk1 (4). Claspin is a chromatin-bound protein, and has been shown to interact with the PNCA complex in the absence of DNA damage (5). Following checkpoint activation it remains chromatin-bound but is released from the PCNA complex and is phosphorylated in an ATR-dependent manner. Phosphorylated claspin interacts with several components of the DNA damage response including BRCA1 (6) and Chk1 (7), leading to ATR-dependent phosphorylation on each of these proteins. Phosphorylated Rad17 has also been shown to bind to and regulate the phosphorylation of claspin (8). It has been proposed that claspin behaves as a tumor suppressor in some cases since down-regulation promotes apoptosis following genotoxic stress (2). Conversely, claspin seems to behave as an oncogene in other instances since overexpression promotes cellular proliferation (6). Upregulated claspin has been suggested to be a sensitive marker of abnormally proliferating cells (9).

**Specificity/Sensitivity:** Claspin Antibody detects endogenous levels of total claspin protein.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to amino acids near the carboxy terminus of human claspin. Antibodies are purified by peptide affinity chromatography.



Western blot analysis of extracts of HEL, SH-SY5Y and NCCIT cells using Claspin Antibody.

### Background References:

- (1) Kumagai, A. and Dunphy, W.G. (2000) *Mol Cell* 6, 839-49.
- (2) Chini, C.C. and Chen, J. (2003) *J Biol Chem* 278, 30057-62.
- (3) Semple, J.I. et al. (2007) *Cell Death Differ*, Epub ahead of print.
- (4) Chini, C.C. et al. (2006) *Oncogene* 25, 4165-71.
- (5) Brondello, J.M. et al. (2007) *Biochem Biophys Res Commun* 354, 1028-33.
- (6) Lin, S.Y. et al. (2004) *Proc Natl Acad Sci U S A* 101, 6484-9.
- (7) Jeong, S.Y. et al. (2003) *J Biol Chem* 278, 46782-8.
- (8) Wang, X. et al. (2006) *Mol Cell* 23, 331-41.
- (9) Tsimaratou, K. et al. (2007) *J Pathol* 211, 331-9.

**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:**  
 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 Detection Pack #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.**

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence F—Flow cytometry E—ELISA D—DELFIATM  
 Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish B—bovine All—all species expected  
 Species enclosed in parentheses are predicted to react based on 100% sequence 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<sup>®</sup>-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<sup>®</sup> 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 cm<sup>2</sup>) 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<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO<sup>®</sup> 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<sup>®</sup> incubation and declines over the following 2 hours.