

Phospho-β-Catenin (Ser45) Antibody

✓ 100 µl
(10 Western mini-blots)



Cell Signaling

TECHNOLOGY®

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

Applications W	Species Cross-Reactivity H, (M, R)	Molecular Wt. 92 kDa	Source Rabbit
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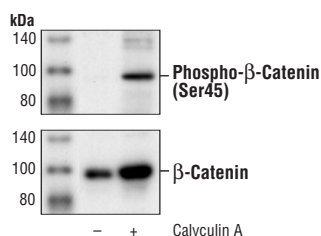
Background: β-catenin is one of the key downstream effectors in the Wnt signaling pathway (1). It has been implicated in two major biological processes of vertebrates: early embryonic development (2) and tumorigenesis (3). GSK-3β may destabilize β-catenin by phosphorylating it at Ser33, 37 and Thr41 (4). Ser45 in β-catenin is phosphorylated by CK1. This likely serves as a priming site for subsequent phosphorylation by GSK-3 (5-7). Stabilizing mutations of these phosphorylation sites in β-catenin have been found in many tumor cell lines (8).

Specificity/Sensitivity: Phospho-β-Catenin (Ser45) Antibody detects endogenous levels of β-catenin only when phosphorylated at serine 45. It does not recognize β-catenin phosphorylated at other sites.

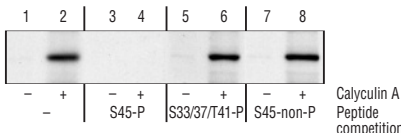
Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with a synthetic phosphopeptide (KLH-coupled) corresponding to residues surrounding Ser45 of human β-catenin. Antibodies are purified by protein A and peptide affinity chromatography.

Background References:

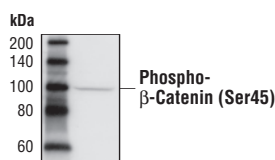
- (1) Cadigan, K.M. and Nusse, R. (1997) *Genes Dev.* 11, 3286–3305.
- (2) Wodarz, A. and Nusse, R. (1998) *Annu. Rev. Cell Dev. Biol.* 14, 59–88.
- (3) Polakis, P. (1999) *Curr. Opin. Genet. Dev.* 9, 15–21.
- (4) Yost, C. et al. (1996) *Genes Dev.* 10, 1443–1454.
- (5) Amit, S. et al. (2002) *Genes Dev.* 16, 1066–1076.
- (6) Lin, C. et al. (2002) *Cell* 108, 837–847.
- (7) Yanagawa, S. et al. (2002) *EMBO J.* 21, 1742.
- (8) Morin, P.J. (1997) *Science* 275, 1787–1790.



Western blot analysis of extracts from HEK293 cells untreated and treated with calyculin A (50 nM for 30 minutes), using Phospho-β-Catenin (upper) and β-Catenin Antibody #9562 (lower).



Western blot analysis of extracts from HEK293 cells untreated and treated with 50 nM calyculin A for 30 minutes, using Phospho-β-Catenin (Ser45) Antibody (lanes 1-2) or the same antibody after preincubation with listed peptides (lanes 3-8). (S45-P is phospho-Ser45 peptide; S33/37/T41-P is phospho-Ser33/37/Thr41 peptide; S45-non-P is nonphospho-Ser45 peptide.)



Western blot analysis of extracts from SW480 cells, using Phospho-β-Catenin (Ser45) Antibody.

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.

Recommended Antibody Dilutions:

Western blotting 1:1000

Companion Products:

Phospho-β-Catenin (Ser33/37/Thr41) Antibody #9561

β-Catenin Antibody #9562

Phospho-β-Catenin (Thr41/Ser45) Antibody #9565

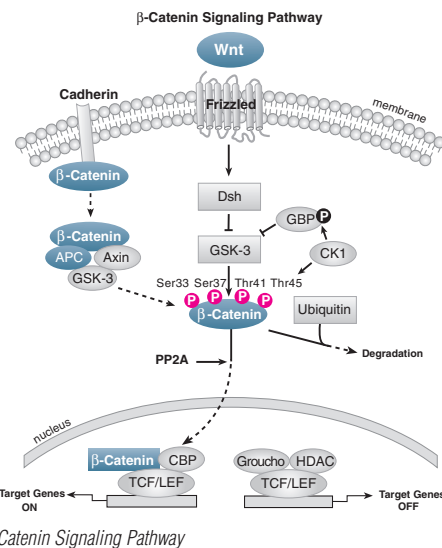
Phototope®-HRP Western Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071

Anti-rabbit IgG, HRP-linked Antibody #7074

Prestained Protein Marker, Broad Range (Premixed Format) #7720

Biotinylated Protein Ladder Detection Pack #7727

LumiGLO® Reagent and 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

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A. Solutions and Reagents

Note: Prepare solutions with Milli-Q or equivalently purified water.

- A1. ■ 1X Phosphate Buffered Saline (PBS)
- A2. ■ 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
- A3. ■ Transfer Buffer:
25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- A4. ■ 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).
- A5. ■ Nonfat Dry Milk (weight to volume [w/v])
- A6. ■ 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%).
- A7. ■ Wash Buffer:
1X TBS, 0.1% Tween-20 (TBS/T)
- A8. ■ Bovine Serum Albumin (BSA)
- A9. ■ 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%).
- A10. ■ 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.
- A11. ■ Prestained Protein Marker, Broad Range (Premixed Format) #7720
- A12. ■ Biotinylated Protein Ladder Detection Pack #7727
- A13. ■ 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.

- B1. Treat cells by adding fresh media containing regulator for desired time.
- B2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- B3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- B4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- B5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- B6. Microcentrifuge for 5 minutes.
- B7. 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.

- B8. 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.

- C1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- C2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- C3. Wash three times for 5 minutes each with 15 ml of TBS/T.
- C4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- C5. Wash three times for 5 minutes each with 15 ml of TBS/T.
- C6. 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.
- C7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D. Detection of Proteins

- D1. 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.

- D2. 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.