

#2910 Store at -20°C

Phospho-Catenin δ -1 (Tyr904) Antibody



- Small 100 μ l (10 Western mini-blots)
- Petite 40 μ l (4 Western mini-blots)

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com

Support ■ 877-678-TECH (8324)
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Web ■ www.cellsignal.com

New 10/07

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	95, 100 kDa	Rabbit

Background: Catenin δ -1 (p120 catenin) has an amino-terminal coiled-coil domain followed by a regulatory domain containing multiple phosphorylation sites and a central Armadillo repeat domain of ten, linked 42 amino-acid repeats. The carboxy-terminal tail has no known function (1). Catenin δ -1 fulfills critical roles in the regulation of cell-cell adhesion as it regulates E-cadherin turnover at the cell surface to determine the level of E-cadherin available for cell-cell adhesion (2). Catenin δ -1 has both positive and negative effects on cadherin-mediated adhesion (3). Actin dynamics are also regulated by catenin δ -1, which modulates RhoA, Rac and cdc42 proteins (1). Analogous to β -catenin, catenin δ -1 translocates to the nucleus although its role at this location is unclear. Many studies show that catenin δ -1 is expressed irregularly or is absent in various types of tumor cells, suggesting that catenin δ -1 may function as a tumor suppressor (4).

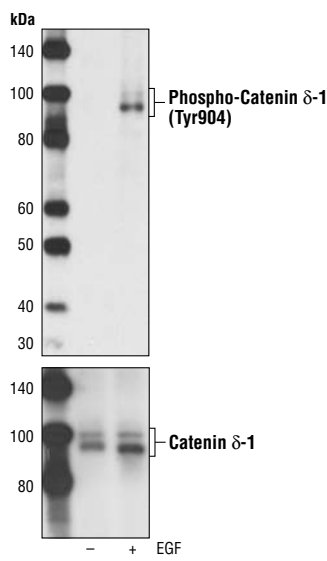
Phosphorylation of Tyr904 on Catenin- δ 1 was identified at Cell Signaling Technology (CST) using PhosphoScan[®], a CST's LC-MS/MS platform for phosphorylation site discovery (5).

Specificity/Sensitivity: Phospho-Catenin δ -1 (Tyr904) Antibody detects endogenous levels of catenin δ -1 protein only when phosphorylated at Tyr904. The antibody might cross react with another overexpressed phospho-tyrosine protein.

Source/Purification: Polyclonal antibodies are produced by immunizing rabbits with synthetic phosphopeptides (KLH-coupled) corresponding to residues surrounding Tyr904 of human/mouse catenin δ -1. Antibodies are purified by peptide affinity chromatography.

Background References:

- (1) Reynolds, A.B. and Rocznik-Ferguson, A. (2004) *Oncogene*. 23, 7947–7956.
- (2) Davis, M. A. et al. (2003) *J Cell Biol.* 163, 525–534.
- (3) Anastasiadis, P.Z. and Reynolds, A.B. (2000) *J Cell Sci.* 113, 1319–1334.
- (4) Thoreson, M.A. and Reynolds, A.B. (2002) *Differentiation*. 70, 583–589.
- (5) Rush, J. et al. (2005) *Nat Biotechnol* 23, 94–101.



Western blot analysis of extracts from overnight serum starved A431 cells, untreated or EGF-treated for 15 minutes, using Phospho-Catenin δ -1 (Tyr904) Antibody (upper) and Catenin δ -1 Antibody #4989 as a loading control (lower).

Entrez-Gene ID # 1500
Swiss-Prot Acc. # O60716

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μ g/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-Catenin δ -1 (Tyr 228) Antibody #2911
- Catenin δ -1 Antibody #4989
- 20X LumiGLO[®] Reagent and 20X Peroxide #7003
- 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 #7727

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—DELFI[®]

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[®]-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.