

**#9961** Store at **-20°C**

# Cadherin-Catenin Antibody Sampler Kit

1 Kit  
 (6 X 40 µl)



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

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**This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.**

Products Included	Product #	Quantity	Mol. Wt.	Isotype
β-Catenin (6B3) Rabbit mAb	9582	40 µl	92 kDa	Rabbit IgG
α-E-Catenin (23B2) Rabbit mAb	3240	40 µl	100 kDa	Rabbit IgG
N-Cadherin Antibody	4061	40 µl	140 kDa	Rabbit IgG
E-Cadherin (24E10) Rabbit mAb	3195	40 µl	135 kDa	Rabbit IgG
P-Cadherin (C13F9) Rabbit mAb	2189	40 µl	120 kDa	Rabbit IgG
Pan-Cadherin (28E12) Rabbit mAb	4073	40 µl	130 to 150 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

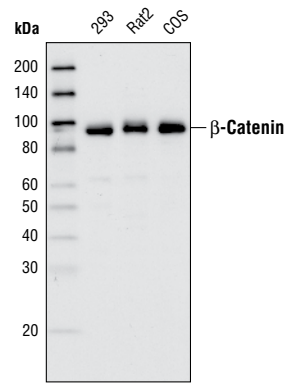
See [www.cellsignal.com](http://www.cellsignal.com) for individual component applications, species cross-reactivity, dilutions and additional application protocols.

**Description:** This Cadherin-Catenin Antibody Sampler kit contains reagents to examine the total protein levels of key proteins found in cell-cell adherens junctions.

**Background:** Adherens junctions are dynamic structures that form cell-cell contacts and are important in development, differentiation, tissue integrity, morphology and cell polarity. They are composed of cadherins that are transmembrane proteins that bind cadherins on adjacent cells in a calcium dependent manner. On the cytoplasmic side of adherens junctions, the cadherins associate with β-catenin, γ-catenin and p120 catenin (δ). β-catenin and γ-catenin associate with α-catenin, which links the cadherin-catenin complex to the actin cytoskeleton (1,2). Recent studies indicate that cancer cells exhibit increased N-cadherin and diminished E-cadherin expression. E-cadherin is considered a suppressor of invasive cancer cell growth and this change in cadherin expression associated with cancer progression is termed the "cadherin switch". β-catenin is one of the key downstream effectors in the Wnt signaling pathway and has been implicated in early embryonic development and tumorigenesis (3-5).

**Specificity/Sensitivity:** Each antibody in the Cadherin-Catenin Antibody Sampler Kit recognizes only its specific target and does not cross-react with other family members. Pan-Cadherin (28E12) Rabbit mAb #4073 detects endogenous levels of total cadherin proteins.

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to the carboxy-terminus of human β-catenin and residues within human N-cadherin and are purified by protein A and peptide affinity chromatography. Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to the amino-terminal sequence of human α-E-catenin, residues near the carboxy terminus of human



Western blot analysis of total cell lysates from 293, Rat2 and COS cells using β-Catenin (6B3) Rabbit mAb #9582.

P-cadherin, residues surrounding 780 of human E-cadherin, and a synthetic peptide corresponding to a conserved region of human N-, R-, E- and P-Cadherin.

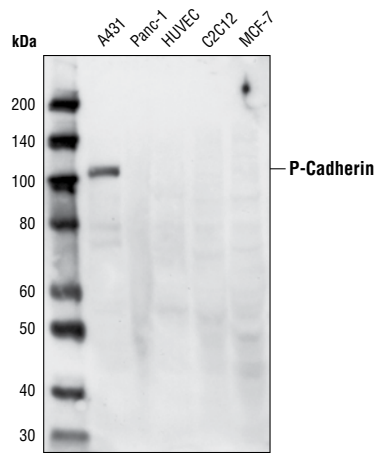
**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibodies.

**Recommended Antibody Dilutions:**  
 Western blotting 1:1000

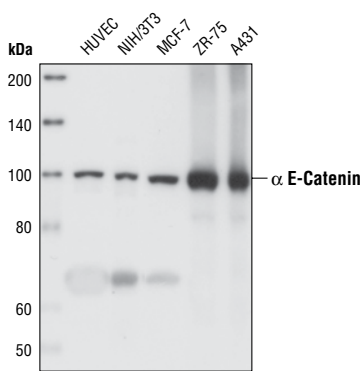
**Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.**

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**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine  
 Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



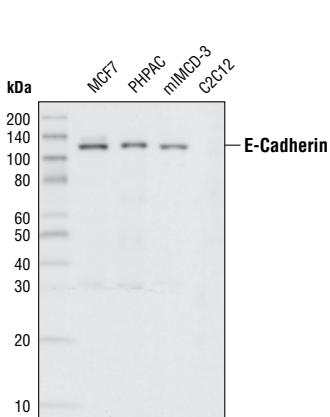
Western blot analysis of extracts from various cell lines using **P-Cadherin (C13F9) Rabbit mAb #2189**.



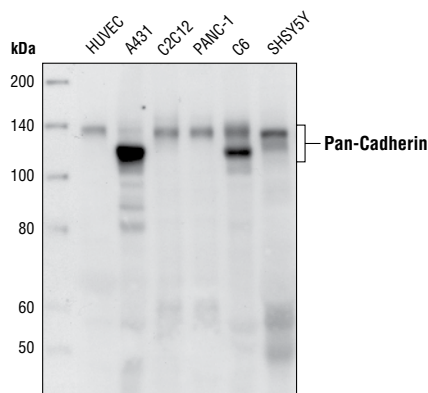
Western blot analysis of extracts from HUVEC, NIH/3T3, MCF-7, ZR-75 and A431 cells using **alpha-E-Catenin (23B2) Rabbit mAb #3240**.

**Background References:**

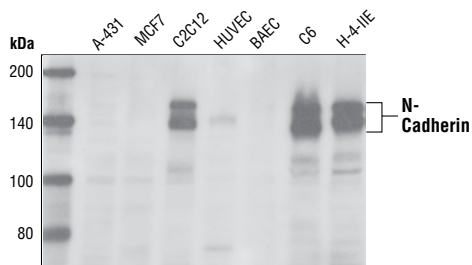
- (1) Wheelock, M.J. and Johnson, K.R. (2003) *Annu. Rev. Cell Dev. Biol.* 19, 207–235.
- (2) Christofori, G. (2003) *EMBO J.* 22, 2318–2323.
- (3) Cadigan, K.M. and Nusse, R. (1997) *Genes Dev.* 11, 3286–3305.
- (4) Wodarz, A. and Nusse, R. (1998) *Annu. Rev. Cell Dev. Biol.* 14, 59–88.
- (5) Polakis, P. (1999) *Curr. Opin. Genet. Dev.* 9, 15–21.



Western blot analysis of extracts from various cell lines using **E-Cadherin (24E10) Rabbit mAb #3195**.



Western blot analysis of extracts from various cell lines using **Pan-Cadherin (28E12) Rabbit mAb #4073**.



Western blot analysis of extracts from various cell lines using **N-Cadherin Antibody #4061**.

## Western Immunoblotting Protocol (Primary Ab 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.