#9961 Store at –20°C

Cadherin-Catenin Antibody Sampler Kit

✓ 1 Kit
(6 X 40 µl)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
β-Catenin (D10A8) XP® Rabbit mAb	8480	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 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 antibody is produced by immunizing animals with a synthetic peptide corresponding to the residues within the amino-terminus of human N-cadherin and is 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 P-cadherin, residues surrounding 780 of human E-cadherin, residues surrounding Pro714 of human ß-catenin protein, and a synthetic peptide corresponding to a conserved region of human N-, R-, E- and P-Cadherin.

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.

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 for a complete listing of recommended companion products.

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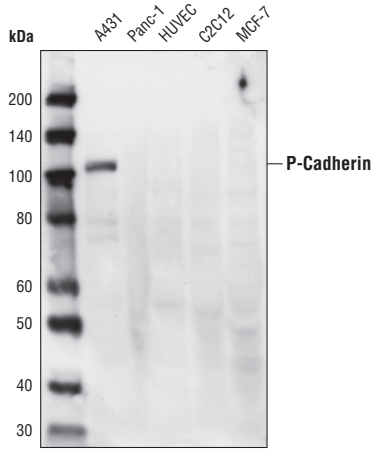
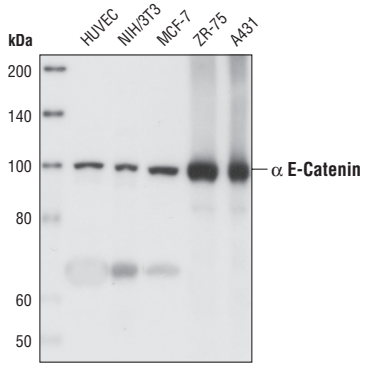
Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

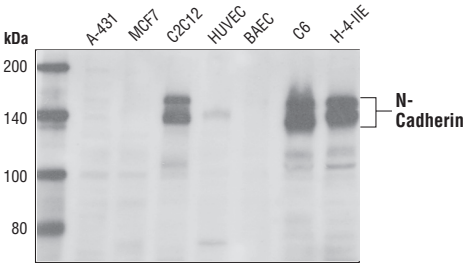
Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



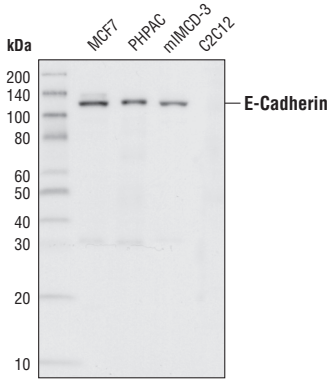
Western blot analysis of extracts from various cell lines using ***P-Cadherin (C13F9) Rabbit mAb #2189****.*



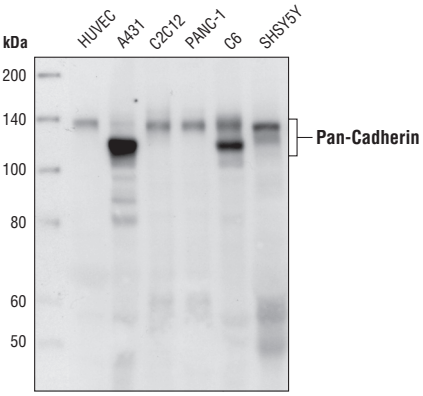
Western blot analysis of extracts from HUVEC, NIH/3T3, MCF-7, ZR-75 and A431 cells using ***α-E-Catenin (23B2) Rabbit mAb #3240.***



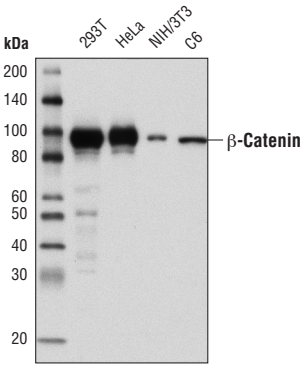
Western blot analysis of extracts from various cell lines using ***N-Cadherin Antibody #4061.***



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 ***β-Catenin (D10A8) XP® Rabbit mAb #8480****.*

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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.

1. 1X Phosphate Buffered Saline (PBS)
2. **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
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **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).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **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%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. **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%).
10. **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.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. **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.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. 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.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. 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.

8. Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. 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.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. 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.

2. 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.

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