

# Pan-Cadherin (28E12) Rabbit mAb

100 µl  
(10 Western mini-blot)

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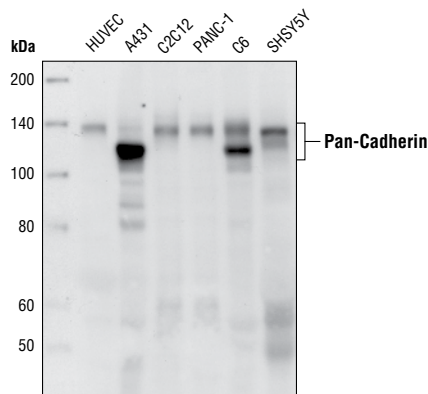
This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity	Molecular Wt.	Source	Isotype
W	H, M, R	130-150 kDa	Rabbit*	IgG

**Background:** Cadherins are a superfamily of trans-membrane glycoproteins that contain cadherin repeats of approximately 100 residues in their extracellular domain. Cadherins mediate calcium-dependent cell-cell adhesion and play critical roles in normal tissue development (1). The classic cadherin subfamily includes N-, P-, R-, B- and E-cadherins as well as about ten other members which are found in adherens junctions (AJ), a cellular structure near the apical surface of polarized epithelial cells. The cytoplasmic domain of classical cadherins interacts with β-catenin, γ-catenin (also called plakoglobin) and p120 catenin. β-catenin and γ-catenin associate with α-catenin, which links the cadherin-catenin complex to the actin cytoskeleton (1,2). Unlike β- and γ-catenin, p120 regulates cadherin adhesive activity and trafficking rather than having a structural role in the junctional complex (1-4). E-cadherin is considered an acting suppressor of invasion and growth of many epithelial cancers (1-3). Recent studies indicate that cancer cells have up-regulated N-cadherin in addition to loss of E-cadherin. This change in cadherin expression is called the "cadherin switch." N-Cadherin cooperates with the FGF receptor, leading to over-expression of MMP-9 and cellular invasion (3). In endothelial cells, VE-cadherin signaling, expression and localization are correlated with vascular permeability and tumor angiogenesis (5,6).

**Specificity/Sensitivity:** Pan-Cadherin (28E12) Rabbit mAb detects endogenous levels of total cadherin proteins.

**Source/Purification:** Monoclonal antibody is produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to a conserved region of human N-, R-, E- and P-Cadherin.



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

**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) Hazan, R.B. et al. (2004) *Ann. NY Acad. Sci.* 1014, 155-163.
- (4) Bryant, D.M. and Stow, J.L. (2004) *Trends Cell Biol.* 14, 427-434.
- (5) Rabascio, C. et al. (2004) *Cancer Res.* 64, 4373-4377.
- (6) Yamaoka-Tojo, M. et al. (2006) *Arterioscler. Thromb. Vasc. Biol.*, Epub ahead of print.

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

**\*Anti-rabbit secondary antibodies must be used to detect this antibody**

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

**Companion Products:**

- Pan-Cadherin Antibody #4068
- VE-Cadherin Antibody #2158
- P-Cadherin Antibody #2130
- N-Cadherin Antibody #4061
- E-Cadherin Antibody #4065
- E-Cadherin (24E10) Rabbit mAb #3195
- 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  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus  
Species enclosed in parentheses are predicted to react based on 100% sequence homology.

F—Flow cytometry E—ELISA D—DELFIATM  
Z—zebra fish B—bovine All—all species expected

## 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®-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 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® (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.