Store at 4°C and –20°C

#7887

Pathscan® Total E-Cadherin Sandwich ELISA Antibody Pair

☑ 1 Kit
(4 X 96 assays)

Cell Signaling TECHNOLOGY®

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New 10/08

Entrez-Gene ID #999
Swiss-Prot Acc. #P12830

Species Cross-Reactivity: H

Description: CST's PathScan® Total E-Cadherin Sandwich ELISA Antibody Pair #7887 is being offered as an economical alternative to our PathScan® Total E-Cadherin Sandwich ELISA Kit #7886. Capture and detection antibodies (100X stocks) and an HRP-conjugated secondary antibody (1000X stock) are supplied. Sufficient reagents are supplied for 4 x 96 well ELISAs. The E-cadherin mouse capture antibody is coated onto a 96 well microplate overnight in PBS. After blocking, cell lysate is added followed by a pan-cadherin rabbit detection antibody and HRP-conjugated, anti-rabbit IgG antibody. HRP substrate (TMB) is then added for color development. The magnitude of the absorbance for this developed color is proportional to the quantity of E-cadherin.

Reagents not supplied:
Phosphate Buffered Saline (PBS-20X) #9808

Phosphate Buffered Saline with Tween-20 (PBST-20X) #9809

Cell Lysis Buffer (10X) #9803

TMB Substrate #7004

STOP Solution #7002

Blocking Buffer: 1X PBS/0.05% Tween-20, 1% BSA

96 Well Microplates**

Microplate Reader

** Antibody Pairs have been validated on Corning® 96 Well Clear Polystyrene High Bind Stripwell™ Microplates (#2592) and Corning® 96 Well EIA/RIA Easy Wash™ Clear Flat Bottom Polystyrene High Bind Microplates (#3369).

Notes: Antibody pairs have been optimized using recommended buffers, reagents, plates and the included protocol. Solutions should be made fresh daily.

Background: Cadherins are a superfamily of transmembrane 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). Expression of P-cadherin, which is normally present in epithelial cells, is also altered in ovarian and other human cancers (7,8).

Products Included	Volume	Cap Color	Storage
E-Cadherin Mouse Capture Antibody (100X)	0.4 ml	Pink	4°C
Pan-Cadherin Rabbit Detection Antibody (100X)	0.4 ml	Blue	4°C
Anti-Rabbit IgG, HRP-Linked Antibody (1000X)	0.04 ml	Yellow	–20°C

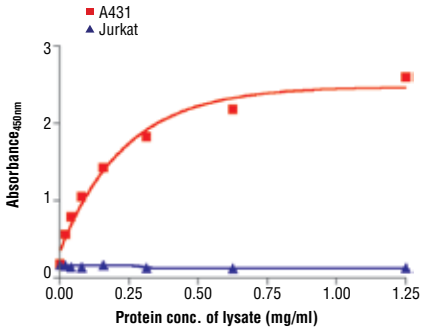

Figure 1. The relationship between the protein concentration of the lysate from A431 (E-cadherin positive) and Jurkat (E-cadherin negative) cells and the absorbance at 450 nm using the PathScan® Total E-Cadherin Sandwich ELISA Antibody Pair is shown.

Storage: E-Cadherin Mouse Capture and Pan-Cadherin Rabbit Detection Antibodies are stored at 4ºC. Anti-Rabbit IgG, HRP-Linked Antibody is stored at -20ºC.

Companion Products:
E-Cadherin (24E10) Rabbit mAb #3195

E-Cadherin Antibody #4065

Anti-rabbit IgG, HRP-linked Antibody #7074

Cell Lysis Buffer (10X) #9803

TMB Substrate #7004

STOP Solution #7002

BSA #9998

Phosphate Buffered Saline (PBS-20X) #9808

Phosphate Buffered Saline with Tween 20 (PBST-20X) #9809

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.* 26, 1991–1997.

(7) Patel, I.S. et al. (2003) *Int. J. Cancer* 106, 172–177.

(8) Sanders, D.S. et al. (2000) *J. Pathol.* 190, 526–530.

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**—zebra fish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

PathScan® Sandwich ELISA Antibody Pair Protocol

A Required Reagents

1. **Coating Buffer:** 1X PBS, (20X PBS #9808)
   3.2 mM Na_2HPO_4, 0.5 mM KH_2PO_4, 1.3 mM KCl, 135 mM NaCl, pH 7.4
2. **Wash Buffer:** 1X PBS/0.05% Tween-20, (20X PBST #9809)
3. **Blocking Buffer:** 1X PBS/0.05% Tween-20, 1% BSA
4. **1X Cell Lysis Buffer:** (10X Cell Lysis Buffer #9803)
   20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM ethylene diamine tetraacetate (EDTA), 1 mM ethylene glycol-bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 µg/ml leupeptin.
5. **TMB Substrate:** (TMB Substrate #7004)
6. **STOP Solution:** (STOP Solution #7002)

NOTE: Reagents should be made fresh daily

B Coating Procedure

1. Rinse microplate with dH_2O. Add 200 µl of dH_2O and discard liquid. Blot on paper towel to make sure wells are dry.
2. Dilute capture antibody 1:100 in PBS. For a single 96 well plate, add 100 µl of Capture Antibody Stock to 9.9 ml PBS. Mix well and add 100 µl/well. Cover plate and incubate overnight at 4°C (17-20 hours).
3. **After overnight coating, gently uncover plate and wash wells:**
   a. Discard plate contents into a receptacle.
   b. Wash 4 times with Wash Buffer, 200 µl each time for each well. For each wash, strike plates on fresh towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
   c. Clean the underside of all wells with a lint-free tissue.
4. Block plates. Add 150 µl of Blocking Buffer/well, cover plate and incubate at 37°C for 2 hours.
5. After blocking, wash plate as in Step 3. Plate is ready to use.

C Preparing Cell Lysates

1. Aspirate media, treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM phenylmethylsulfonyl fluoride (PMSF) to each plate (10 cm diameter plate) and incubate the plate on ice for 5 minutes.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 minutes at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

D Test Procedure

1. Lysates can be used undiluted or diluted in Blocking Buffer. 100 µl of lysate is added per well. Cover plate and incubate at 37°C for 2 hours.
2. Wash plate as in Coating Procedure, Step 3.
3. Dilute detection antibody 1:100 in Blocking Buffer. For a single 96 well plate, add 100 µl of Detector Antibody Stock to 9.9 ml of Blocking Buffer. Mix well and add 100 µl/well. Cover plate and incubate at 37°C for 1 hour.
4. Plate is washed as in Coating Procedure, Step 3.
5. Secondary antibody, either, anti-mouse or anti-rabbit-HRP, is diluted 1:1000 in Blocking Buffer. For a single 96 well plate, add 10 µl of secondary antibody stock to 9.99 ml of Blocking Buffer. Mix well and add 100 µl/well. Cover and incubate at 37°C for 30 minutes.
6. Wash plate as in Coating Procedure, Step 3.
7. Add 100 µl of TMB Substrate per well. Cover and incubate at 37°C for 10 minutes.
8. Add 100 µl of STOP Solution per well.
9. Read plate on a microplate reader at Absorbance 450 nm.