

# **Human/Mouse E-Cadherin Antibody**

Antigen Affinity-purified Polyclonal Goat IgG Catalog Number: AF648

DESCRIPTION			
Species Reactivity	Human/Mouse		
Specificity	Detects human E-Cadherin in direct ELISAs and Western blots.		
Source	Polyclonal Goat IgG		
Purification	Antigen Affinity-purified		
Immunogen	Mouse myeloma cell line NS0-derived recombinant human E-Cadherin Asp155-lle707 Accession # P12830		
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.		

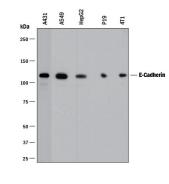
## APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.

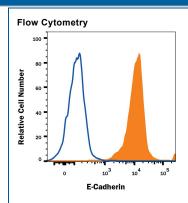
	Recommended Concentration	Sample	
Dual RNAscope ISH-IHC Compatible	0.3-15 μg/mL	See Below	
Western Blot	0.5 μg/mL	See Below	
Flow Cytometry	0.25 μg/10 <sup>6</sup> cells	See Below	
Immunocytochemistry	5-15 μg/mL	See Below	
Immunohistochemistry	0.3-15 μg/mL	See Below	
Simple Western	5 μg/mL	See Below	
CyTOF-ready	Ready to be labeled with conjugation.	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could inte with conjugation.	

#### DATA

#### Western Blot

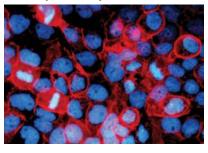


**Detection of Human and Mouse** E-Cadherin by Western Blot. Western blot shows lysates of A431 human epithelial carcinoma cell line, A549 human lung carcinoma cell line, HepG2 human hepatocellular carcinoma cell line, P19 mouse embryonal carcinoma cell line, and 4T1 mouse breast cancer cell line. PVDF membrane was probed with 0.5 µg/mL of Goat Anti-Human/Mouse E-Cadherin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF648) followed by HRPconjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for E-Cadherin at approximately 110 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.



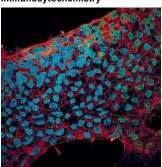
Detection of E-Cadherin in MCF-7 Human Cell Line by Flow Cytometry. MCF-7 human breast cancer cell line was stained with Goat Anti-Human/Mouse E-Cadherin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF648, filled histogram) or isotype control antibody (Catalog # AB-108-C, open histogram), followed by Allophycocyanin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0108). View our protocol for Staining Membrane-associated Proteins.

## Immunocytochemistry



E-Cadherin in Human Epidermoid Carcinoma Cells. E-Cadherin was detected in immersion fixed human epidermoid carcinoma cells using 10 µg/mL Goat Anti-Human/Mouse E-Cadherin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF648) for 3 hours at room temperature. Cells were stained with the NorthernLights™ 557conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

## Immunocytochemistry



E-Cadherin and SOX2 in BG01V Human Stem Cells, E-Cadherin and SOX2 were detected in BG01V human embryonic stem cells using 10 µg/mL Goat Anti-Human/Mouse E-Cadherin Antigen Affinitypurified Polyclonal Antibody (Catalog # AF648) and 10 µg/mL Human/Mouse SOX2 Monoclonal Antibody (Catalog # MAB2018). Cells were incubated with primary antibodies for 3 hours at room temperature. Cells were stained for E-Cadherin using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (green; Catalog # NL001) and for SOX2 using the NorthernLights 493-conjugated Anti-Mouse Secondary Antibody (red; Catalog # NL009). Cells were counterstained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

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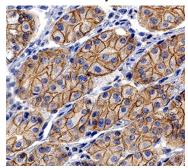




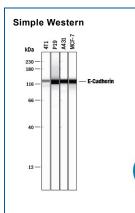
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#### Immunohistochemistry

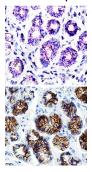


E-Cadherin in Human Stomach. E-Cadherin was detected in immersion fixed paraffin-embedded sections of human stomach using Goat Anti-Human/Mouse E-Cadherin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF648) at 0.3 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC004). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cell membrane and cytoplasm in gastric glands. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.



Detection of Human and Mouse E-Cadherin by Simple Western<sup>™</sup>. Simple Western lane view shows lysates of 4T1 mouse breast cancer cell line, P19 mouse embryonal carcinoma cell line, A431 human epithelial carcinoma cell line, and MCF-7 human breast cancer cell line, loaded at 0.2 mg/mL. A specific band was detected for E-Cadherin at approximately 128 kDa (as indicated) using 5  $\mu g/mL$  of Goat Anti-Human/Mouse E-Cadherin Antigen Affinitypurified Polyclonal Antibody (Catalog # AF648) followed by 1:50 dilution of HRPconjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

#### **Dual RNAscope ISH-IHC Compatible**



E-Cadherin in Human Stomach Using Dual RNAscope® ISH and IHC. E-Cadherin mRNA was detected in formalin-fixed paraffinembedded tissue sections of human stomach probed with ACD RNAScope® Probe (Catalog # 311091) and stained using ACD RNAscope® 2.5 HD Detection Reagents-Red (top image, Catalog # 32260). Adjacent tissue section was processed for immunohistochemistry using R&D Systems Goat Anti-Human E-Cadherin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF648) at 0.3 ug/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte HRP Polymer Antibody (R&D Systems, Catalog # VC004) and DAB chromogen (lower image, yellow-brown). Tissues were counterstained with hematoxylin (blue).

## PREPARATION AND STORAGE

Reconstitution Reconstitute at 0.2 mg/mL in sterile

Shipping

The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. \*Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C

## Stability & Storage

## Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 12 months from date of receipt, -20 to -70 °C as supplied
- 1 month, 2 to 8 °C under sterile conditions after reconstitution.
- 6 months, -20 to -70 °C under sterile conditions after reconstitution.

### BACKGROUND

Epithelial (E)-Cadherin (ECAD), also known as cell-CAM120/80 in the human, uvomorulin in the mouse, Arc-1 in the dog, and L-CAM in the chicken, is a member of the Cadherin family of cell adhesion molecules. Cadherins are calcium-dependent transmembrane proteins which bind to one another in a homophilic manner. On their cytoplasmic side, they associate with the three catenins,  $\alpha$ ,  $\beta$ , and  $\gamma$  (plakoglobin). This association links the cadherin protein to the cytoskeleton. Without association with the catenins, the cadherins are non-adhesive. Cadherins play a role in development, specifically in tissue formation. They may also help to maintain tissue architecture in the adult. E-Cadherin may also play a role in tumor development, as loss of E-Cadherin has been associated with tumor invasiveness. E-Cadherin is a classical cadherin molecule. Classical cadherins consist of a large extracellular domain which contains DXD and DXNDN repeats responsible for mediating calcium-dependent adhesion, a single-pass transmembrane domain, and a short carboxy-terminal cytoplasmic domain responsible for interacting with the catenins. E-Cadherin contains five extracellular calcium-binding domains of approximately 110 amino acids each.

### References:

- 1. Bussemakers, M.J.G. et al. (1993) Mol. Biol. Reports 17:123.
- 2. Overduin, M. et al. (1995) Science 267:386.
- 3. Takeichi, M. (1991) Science 251:1451.

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