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Product datasheet for AM32138PU-N

E Cadherin (CDH1) Mouse Monoclonal Antibody [Clone ID: 5H9]

Product data:

Product Type:	Primary Antibodies
Clone Name:	5H9
Applications:	IF, IHC, WB
Recommended Dilution:	**Western blot.** **Immunocytochemistry.** **Immunohistochemistry on Frozen sections:** Use a PBS buffer containing 0.1 mM $CaCl_2$ and 0.1 mM $MgCl_2$. **Immunohistochemistry on Paraffin sections:** Use a pretreatment step of 15 minutes incubation in TRIS-EDTA buffer pH 9 in a microwave. *Recommended Dilutions:* 1/50–1/100 for immunohistochemistry with ABC as detection reagent, and 1/100-1/500 for immunoblotting. *Recommended Positive Control:* Cell line MCF-7, Human small Intestine.
Reactivity:	Human
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Affinity purified 80 kDa extracellular fragments of E-Cadherin derived from tryptic digestion of A-431 Human vulva carcinoma cells. **Epitope:** Extracellular domain. **Myeloma:** P3x63-Ag8,653.
Specificity:	The antibody 5H9 recognizes both the 120 kDa E-Cadherin and its 80 kDa trypsin-resistant extracellular part.
Formulation:	PBS State: Purified State: Liquid purified IgG fraction Preservative: 0.09% Sodium Azide
Concentration:	lot specific
Conjugation:	Unconjugated

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Storage: Store undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer.
Avoid repeated freezing and thawing.

Stability: Shelf life: one year from despatch.

Gene Name: cadherin 1

Database Link: Entrez Gene 999 Human
P12830

Background: Cadherins constitute a family of transmembrane glycoproteins involved in Ca^{2+}-dependent cell-cell interactions. The members of this family are differentially expressed in various tissues. They function in the maintenance of tissue integrity and morphogenesis. Cadherins are divided into type I and type II subgroups. Type I cadherins include epithelial cadherin (E-cadherin, cadherin-1 or uvomorulin), neural cadherin (N-cadherin or cadherin-2), placental cadherin (P-cadherin or cadherin-3) and retinal cadherin (R-cadherin or cadherin-4), whereas kidney cadherin (K-cadherin or cadherin-6) and osteoblast cadherin (OB-cadherin or cadherin-11) are type II cadherins.
One of the best characterized cadherins is E-cadherin, a 120 kD transmembrane glycoprotein consisting of an 80 kD extracellular and a 40 kD transmembrane and cytoplasmic part. The extracellular domains of E-cadherin are responsible for calcium binding which allows for homophilic interaction with other E-cadherin molecules on the same cell and neighbouring cells. In addition, E-cadherin can interact heterophilically with integrin $\alpha_E\beta_7$.
The cytoplasmic domain of E-cadherin is linked to the actin cytoskeleton through the associated cytoplasmic catenin proteins, thus establishing a complex localized to adherens junctions. In carcinomas E-cadherin is frequently downregulated, which is consistent with its function of an invasion suppressor in normal epithelia.
One of the epithelial cell adhesion molecules, E-Cadherin, plays an important role in the formation of cell-cell contacts in epithelia irrespective their origin form ecto-, meso- or endodermal tissue. This early adhesion event between epithelial cells is a prerequisite for the assembly of all elements of the junctional complex. Furthermore, ECadherin plays a crucial role in the maintenance of the epithelial junctional complex and is as such an important molecule in maintaining epithelial integrity. Over 90% of the malignant tumors are carcinomas. One of the prerequisites for the release of carcinoma cells from the primary site might be a defect in intercellular adhesion mediated by the absence of E-Cadherin expression. Therefore, the expression of E-Cadherin might be an important parameter for the determination of the invasive potential of epithelial neoplasms, and for the transition of a benign to a malignant neoplasm.

Synonyms: Epithelial cadherin, E-cadherin, Uvomorulin, CAM 120/80, CDH1, CDHE, UVO

Note:

Protocol: **Indirect Immunoperoxidase Staining On Formalin-Fixed Paraffin-Embedded Tissue - Microwave Treatment.**

1. Fix paraffin sections onto silanated oder polylysin-coated slides.
2. Dry overnight at 58°C.
3. Deparaffinize: dewax in xylol for 3 x 3 min; rehydrate in decreasing grades of ethanol: absolute,
96%, 70%, 50%, and dest. water for 3 min ea.
4. Microwave treatment incubate in plastic cuvette containing cold 10 mM Tris-EDTA buffer (50 mM Tris, 1 mM EDTA, pH 9.0) 3 x 5 min at 600 Watt in a microwave oven; let cool down after complete treatment to
room temperature (15 min).
5. From this step onward it is essential that the sections do not dry out.
6. Rinse in dest. water and 2 x 5 min in PBS.
7. Optional:
Block endogenous peroxidase with 0.6% H_2O_2/40% methanol-PBS for 30 min.
8. Rinse in PBS for 2 x 5 min.
9. Cover sections with 10 % heat-inactivated horse serum in PBS for 1 h (or alternatively with 5 % normal serum of the same species as the secondary antibody); depending on background staining, this step can be omitted or the serum concentration can be increased. Decant excessserum after incubation.[1)]
10. Incubation with optimal dilution of primary antibody (optimal dilution should be tested individually in each laboratory; start with a dilution of 1:50).
11. Rinse in PBS for 3 x 5 min.
12. Detection system: ABC method (e.g. Vector Laboratories); follow kit instructions; Counterstain with methyl green or hematoxylin
13. Dehydrate in increasing grades of ethanol, clear with xylol and mount with mounting medium.

Indirect Immunoperoxidase Staining On Frozen Sections.

1. Fix sections on clean glass slides
2. Fixation in cold acetone or acetone/methanol for 5-10 min.
3. Let dry at RT
4. Rinse for 2 x 5 min in PBS
5. Optional: Block endogenous peroxidase with 0.6% H_2O_2/40% methanol-PBS for 30 min.
6. Rinse in PBS for 2 x 5 min.
7. Follow steps 10 -13 as given above.

[1)] To reduce background, 2% skim milk powder may be added to the horse serum and all following
incubations.

Product images:

Immunohistochemistry on Paraffin-Embedded Sections of small intestine.