Anti-E-Cadherin Antibody [SY0287]

ET1607-75

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 97 kDa
Clone number:	SY0287

Description: Cadherin-1 (not to be confused with the APC/C activator protein CDH1) also known as CAM 120/80 or epithelial cadherin (E-cadherin) or uvomorulin is a protein that in humans is encoded by the CDH1 gene. Mutations are correlated with gastric, breast, colorectal, thyroid, and ovarian cancers. CDH1 has also been designated as CD324 (cluster of differentiation 324). It is a tumor suppressor gene. E-cadherin (epithelial) is the most well-studied member of the cadherin family. It consists of 5 cadherin repeats (EC1 ~ EC5) in the extracellular domain, one transmembrane domain, and an intracellular domain that binds p120-catenin and beta-catenin. The intracellular domain contains a highly-phosphorylated region vital to beta-catenin binding and, therefore, to E-cadherin function. In epithelial cells, E-cadherin-containing cell-to-cell junctions are often adjacent to actin-containing filaments of the cytoskeleton. E-cadherin is first expressed in the 2-cell stage of mammalian development, and becomes phosphorylated by the 8-cell stage, where it causes compaction.

Immunogen: Synthetic peptide within human E-Cadherin aa 580-630 (Extracellular).

Positive control: MCF7 cell lysate, MDA-MB-231 cell lysate, HT-29 cell lysate, HCT 116 cell lysate, A431 cell lysate, Caco-2 cell lysate, HT-29, MCF-7, human lung carcinoma tissue, human colon tissue, human breast cancer tissue.

Subcellular location: Cell junction, Cell membrane, Endosome, Golgi apparatus.

Database links: SwissProt: P12830 Human

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:2,000
IF-Tissue	1:50-1:200
IHC-P	1:200
FC	1:1,000
IP	Use at an assay dependent concentration.

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

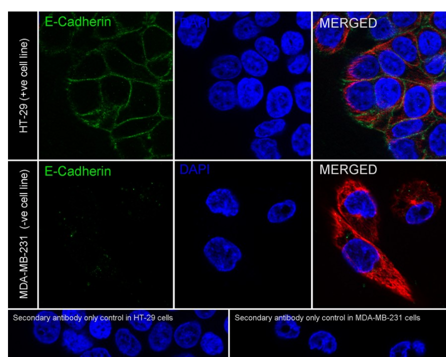

Fig1: Immunocytochemistry analysis of HT-29 (positive) and MDA-MB-231 (negative) cells labeling E-Cadherin with Rabbit anti-E-Cadherin antibody (ET1607-75) at 1/2,000 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-E-Cadherin antibody (ET1607-75) at 1/2,000 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

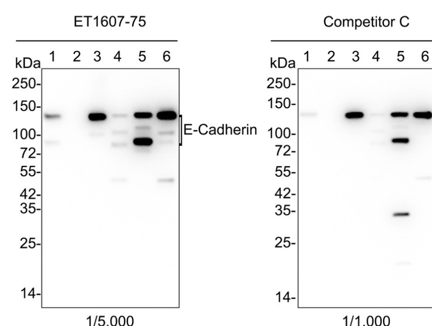

Fig2: Western blot analysis of E-Cadherin on different lysates with Rabbit anti-E-Cadherin antibody (ET1607-75) at 1/5,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: MCF7 cell lysate
Lane 2: MDA-MB-231 cell lysate (negative)
Lane 3: HT-29 cell lysate
Lane 4: HCT 116 cell lysate
Lane 5: A431 cell lysate
Lane 6: Caco-2 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 97 kDa
Observed band size: 80~120 kDa

Exposure time: 43 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1607-75) at 1/5,000 dilution and competitor's antibody at 1/1,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:50,000 dilution was used for 1 hour at room temperature.

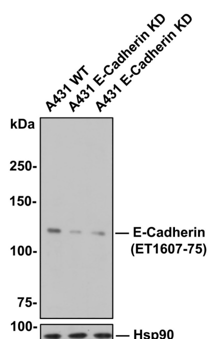

Fig3: All lanes: Western blot analysis of E-Cadherin with anti-E-Cadherin antibody (ET1607-75) at 1:500 dilution.
Lane 1: Wild-type A431 whole cell lysate (10 µg).
Lane 2/3: E-Cadherin knockdown A431 whole cell lysate (10 µg).

ET1607-75 was shown to specifically react with E-Cadherin in wild-type A431 cells. Weakened bands were observed when E-Cadherin knockdown samples were tested. Wild-type and E-Cadherin knockdown samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary antibody (ET1607-75, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG-HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

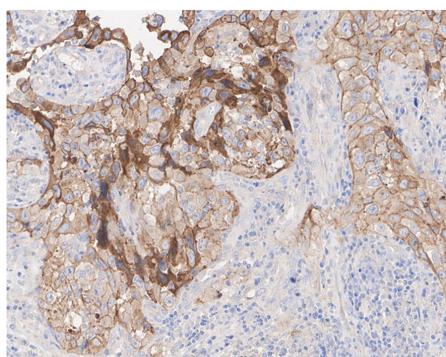

Fig4: Immunohistochemical analysis of paraffin-embedded human lung carcinoma tissue with Rabbit anti-E-Cadherin antibody (ET1607-75) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1607-75) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

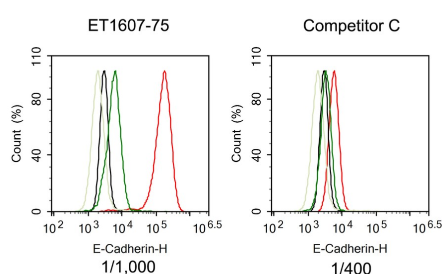

Fig5: Flow cytometric analysis of HT-29 (positive, red) and MDA-MB-231 (negative, green) cells labeling E-Cadherin.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1607-75, red) at 1/1,000 dilution and competitor's antibody (red) at 1/400 dilution, compared with Rabbit IgG Isotype Control (HT-29 black, MDA-MB-231 light green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃.

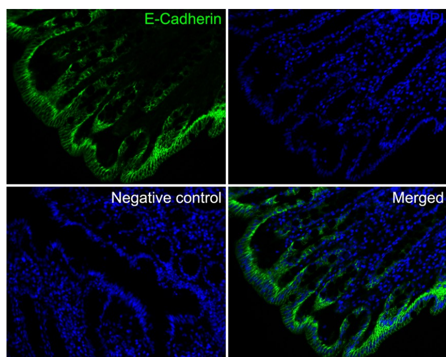

Fig6: Immunofluorescence analysis of paraffin-embedded human colon tissue labeling E-Cadherin with Rabbit anti-E-Cadherin antibody (ET1607-75) at 1/50 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1607-75, green) at 1/50 dilution overnight at 4 ℃, washed with PBS.

Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with 33258 (blue).

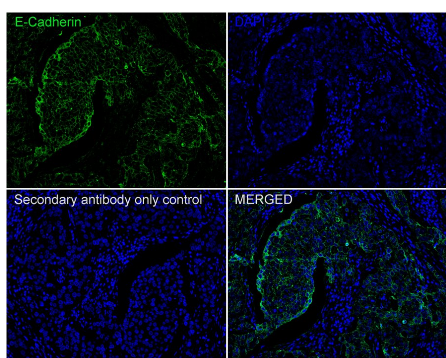

Fig7: Immunofluorescence analysis of paraffin-embedded human breast cancer tissue labeling E-Cadherin with Rabbit anti-E-Cadherin antibody (ET1607-75) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1607-75, green) at 1/200 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Su B et al. Diallyl disulfide suppresses epithelial-mesenchymal transition, invasion and proliferation by downregulation of LIMK1 in gastric cancer. Oncotarget 7:10498-512 (2016).
2. Schmidt TP et al. Identification of E-cadherin signature motifs functioning as cleavage sites for Helicobacter pylori HtrA. Sci Rep 6:23264 (2016).