Anti-E-Cadherin Antibody [PSH13-75]

HA723564

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-Fr, IHC-P, IF-Cell, FC, IP
Molecular Wt:	Predicted band size: 98 kDa
Clone number:	PSH13-75

Description: Cadherin-1 or Epithelial cadherin (E-cadherin), (not to be confused with the APC/C activator protein CDH1) 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. Cadherin-1 is a classical member of the cadherin superfamily. The encoded protein is a calcium-dependent cell–cell adhesion glycoprotein composed of five extracellular cadherin repeats, a transmembrane region, and a highly conserved cytoplasmic tail. Mutations in this gene are correlated with gastric, breast, colorectal, thyroid, and ovarian cancers. Loss of function is thought to contribute to progression in cancer by increasing proliferation, invasion, and/or metastasis. The ectodomain of this protein mediates bacterial adhesion to mammalian cells, and the cytoplasmic domain is required for internalization. Identified transcript variants arise from mutation at consensus splice sites.

Immunogen: Recombinant protein within mouse E-Cadherin aa 157-709.

Positive control: 4T1 cell lysate, Mouse small intestine tissue lysate, Mouse colon tissue lysate, Mouse pancreas tissue lysate, Rat pancreas tissue lysate, Rat lung tissue lysate, Rat colon tissue lysate, MCF7 cell lysate, HT-29 cell lysate, Caco-2 cell lysate, mouse colon tissue, rat colon tissue, MCF7, 4T1.

Subcellular location: Cell junction, adherens junction, Cell membrane, Endosome, Golgi apparatus, trans-Golgi network, Cytoplasm, Cell junction, desmosome.

Database links: SwissProt: P12830 Human | P09803 Mouse | Q9R0T4 Rat

Recommended Dilutions:

WB	1:5,000
IHC-Fr	1:500
IHC-P	1:5,000
IF-Cell	1:100-1:500
FC	1:1,000
IP	1-2μg/sample

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

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

Purity: Protein A affinity purified.

Images

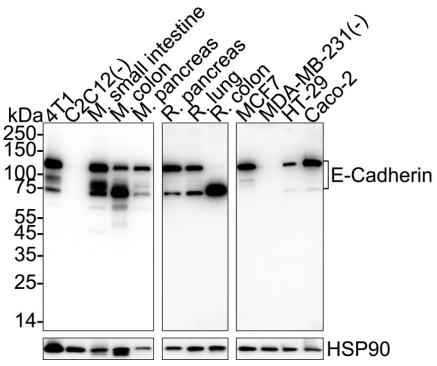

Fig1: Western blot analysis of E-Cadherin on different lysates with Rabbit anti-E-Cadherin antibody (HA723564) at 1/5,000 dilution.

Lane 1: 4T1 cell lysate (20 µg/Lane)
Lane 2: C2C12 cell lysate (negative) (20 µg/Lane)
Lane 3: Mouse small intestine tissue lysate (30 µg/Lane)
Lane 4: Mouse colon tissue lysate (30 µg/Lane)
Lane 5: Mouse pancreas tissue lysate (30 µg/Lane)
Lane 6: Rat pancreas tissue lysate (30 µg/Lane)
Lane 7: Rat lung tissue lysate (30 µg/Lane)
Lane 8: Rat colon tissue lysate (30 µg/Lane)
Lane 9: MCF7 cell lysate (20 µg/Lane)
Lane 10: MDA-MB-231 cell lysate (negative) (20 µg/Lane)
Lane 11: HT-29 cell lysate (20 µg/Lane)
Lane 12: Caco-2 cell lysate (20 µg/Lane)

Predicted band size: 98 kDa
Observed band size: 75-130 kDa

Exposure time: Lane 1-5: 10 seconds; Lane 3: 1 minute 16 seconds; ECL: K1801;

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 (HA723564) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

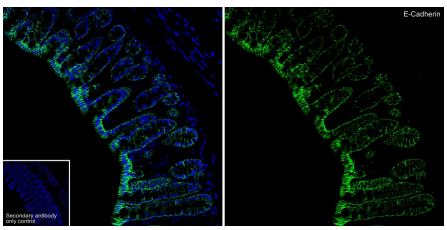

Fig2: Application: IHC-Fr

Species: Mouse

Site: colon

Sample: Frozen section

Antibody concentration: 1/500

Antigen retrieval: Not required

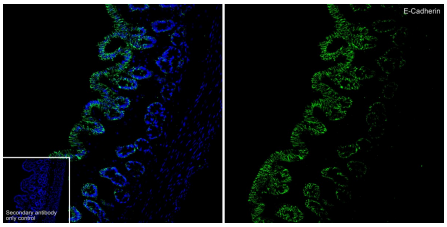

Fig3: Application: IHC-Fr

Species: Rat

Site: colon

Sample: Frozen section

Antibody concentration: 1/500

Antigen retrieval: Not required

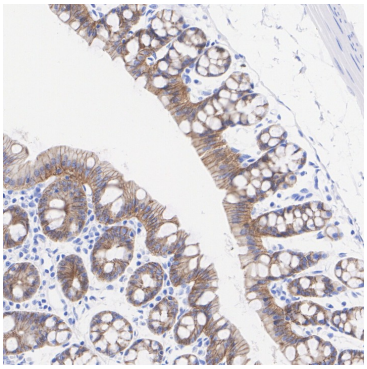

Fig4: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-E-Cadherin antibody (HA723564) at 1/5,000 dilution.

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 (HA723564) at 1/5,000 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.

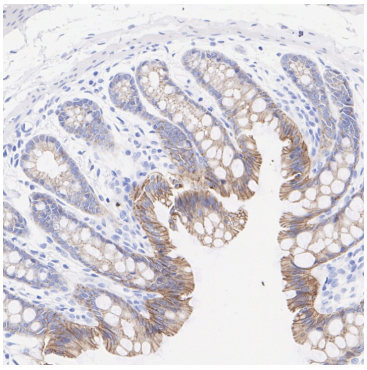

Fig5: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-E-Cadherin antibody (HA723564) at 1/5,000 dilution.

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 (HA723564) at 1/5,000 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.

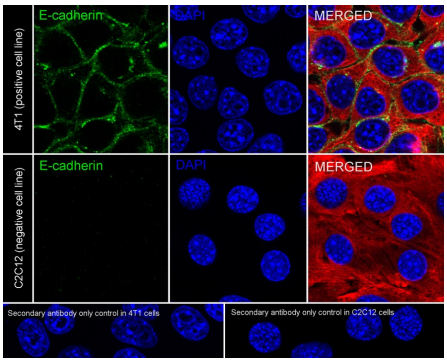

Fig6: Immunocytochemistry analysis of 4T1 (positive) and C2C12 (negative) labeling E-Cadherin with Rabbit anti-E-Cadherin antibody (HA723564) at 1/500 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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 (HA723564) at 1/500 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 (HA601187, 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.

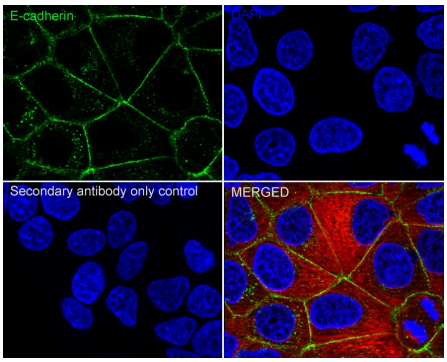

Fig7: Immunocytochemistry analysis of MCF7 cells labeling E-Cadherin with Rabbit anti-E-Cadherin antibody (HA723564) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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 (HA723564) at 1/100 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 (HA601187, 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.

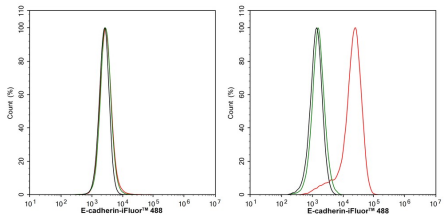

Fig8: Flow cytometric analysis of C2C12 (left, negative) and 4T1 (right, positive) cells labeling E-Cadherin.

Cells were washed twice with cold PBS and resuspend. Then stained with the primary antibody (HA723564, 1/1,000) (red) compared with Rabbit IgG Isotype Control (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℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

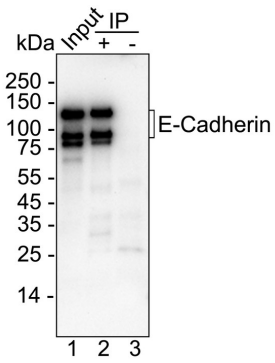

Fig9: E-Cadherin was immunoprecipitated from 0.2 mg 4T1 cell lysate with HA723564 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA723564 at 1/5,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: 4T1 cell lysate (input)
Lane 2: HA723564 IP in 4T1 cell lysate
Lane 3: Rabbit IgG instead of HA723564 in 4T1 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 46 seconds; ECL: K1801

Background References

1. Balamurugan K et al. Stabilization of E-cadherin adhesions by COX-2/GSK3beta signaling is a targetable pathway in metastatic breast cancer. JCI Insight. 2023 Mar
2. Kielbik M et al. E-Cadherin Expression in Relation to Clinicopathological Parameters and Survival of Patients with Epithelial Ovarian Cancer. Int J Mol Sci. 2022 Nov