abcam

ab76055

Anti-E-Cadherin/CD324 antibody [M168] - C-terminal

Anti-E-Cadherin/CD324 antibody [M168] - C-terminal (ab76055) is a mouse monoclonal antibody detecting E-Cadherin/CD324 in **Western Blot, Flow Cytometry (Intra), IHC-P**. Suitable for **Human**.

- Over 400 publications
- Trusted since 2009

Lab Essentials

Key facts

Isotype	IgG1
Host species	Mouse
Storage buffer	Preservative: 0.05% Sodium azide Constituents: PBS, 50% Glycerol (glycerin, glycerine), 0.1% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	Recombinant Fragment Protein within Mouse Cdh1. The exact immunogen used to generate this antibody is proprietary information. Database link P09803
Clone number	M168
Purification technique	Affinity purification Protein A
Specificity	ab76055 does not cross react with VE Cadherin or N Cadherin.
Light chain type	kappa

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	5 µg/mL
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

ICC

Tested

Species	Human
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1/100 - 1/1000
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/100
Notes	ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

Target data

See full target information E-Cadherin/CD324

Function Cadherins are calcium-dependent cell adhesion proteins (PubMed:11976333). They preferentially interact with themselves in a homophilic manner in connecting cells; cadherins may thus contribute to the sorting of heterogeneous cell types. CDH1 is

involved in mechanisms regulating cell-cell adhesions, mobility and proliferation of epithelial cells (PubMed:11976333). Promotes organization of radial actin fiber structure and cellular response to contractile forces, via its interaction with AMOTL2 which facilitates anchoring of radial actin fibers to CDH1 junction complexes at the cell membrane (By similarity). Plays a role in the early stages of desmosome cell-cell junction formation via facilitating the recruitment of DSG2 and DSP to desmosome plaques (By similarity). Has a potent invasive suppressor role. It is a ligand for integrin alpha-E/beta-7 (By similarity). E-Cad/CTF2 promotes non-amyloidogenic degradation of Abeta precursors. Has a strong inhibitory effect on APP C99 and C83 production (By similarity). (Microbial infection) Does not function as a receptor for L.monocytogenes internalin A (InlA); mutating a single surface-exposed residue confers receptor activity to this protein and promotes uptake of the bacteria.

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

What is this antibody validated in?
Anti-E Cadherin antibody [M168] - C-terminal (ab76055) is a mouse monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunohistochemistry (IHC-P) in Human samples.

What is the molecular weight of E Cadherin?
Anti-E Cadherin [M168] - C-terminal (ab76055) specifically detects a band for E Cadherin (UniProt: P09803) at a molecular weight of 97kDa.

Trusted by the scientific community
Anti-E Cadherin [M168] - C-terminal (ab76055) was first used in a scientific publication in 2009 and has been cited over 400 times in peer-reviewed journals.

Reviewed by scientists
Anti-E Cadherin [M168] - C-terminal (ab76055) has over 15 independent reviews from customers.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	E-Cadherin sometimes called CDH1 or Cadherin-1 is a protein that plays a role in cell-to-cell adhesion. This transmembrane protein has a molecular weight of approximately 120 kDa. E-Cadherin is mainly expressed in epithelial tissues of various organs including the skin and gut. Its adhesive function is made possible by its extracellular domain that facilitates homophilic binding between cells contributing to the maintenance of tissue architecture and cellular integrity.
Biological function summary	E-Cadherin participates in establishing and maintaining adherens junctions which are vital for tissue structure. E-Cadherin operates as a core component of the cadherin-catenin complex which links the protein to the actin cytoskeleton. Through this linkage E-Cadherin plays an important role in signaling pathways that influence cellular growth and differentiation. The protein's ability to mediate intercellular connections also regulates cellular motility and supports basic aspects of cell behavior in epithelial tissues.
Pathways	E-Cadherin influences both the Wnt signaling pathway and the epithelial-to-mesenchymal transition (EMT). Within the Wnt signaling pathway E-Cadherin partners with B-catenin a significant player in transcription regulation and cell signaling. Disruption in E-Cadherin's adhesive functionality can lead to increased B-catenin availability affecting downstream transcriptional control. In the EMT process E-Cadherin loss characterizes an important step in which cells gain migratory and invasive properties typically seen during metastasis in cancer progression.
Associated diseases and disorders	E-Cadherin's role is prominent in cancer particularly in the context of gastric and breast cancers. Mutations or altered expression of E-Cadherin lead to diminished cell adhesion promoting tumorigenesis and metastatic spread. In gastric cancer for instance mutated E-Cadherin often associates with loss of epithelial function facilitating cancer cell dissemination. Additionally its loss or dysfunction may correlate with proteins such as B-catenin further impacting cancer progression and pathology.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

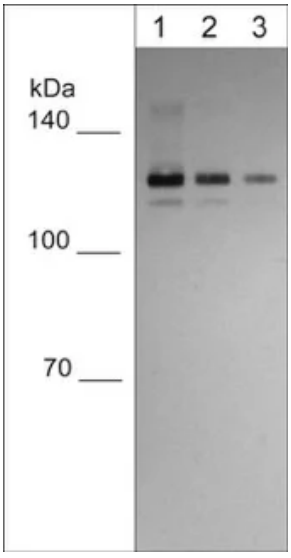
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

8 product images



Western blot - Anti-E-Cadherin/CD324 antibody [M168] - C-terminal (ab76055)

E-Cadherin/CD324 Western blot staining of A431 (Human epidermoid carcinoma cell line) denatured whole cell lysate using mouse Anti-E-Cadherin/CD324 antibody

Blocked and probed in the presence of 5% milk, and the image was captured using chemiluminescence and film.
Lane 1:
Western blot - Anti-E-Cadherin/CD324 antibody [M168] - C-terminal (ab76055) at 1/1000 dilution

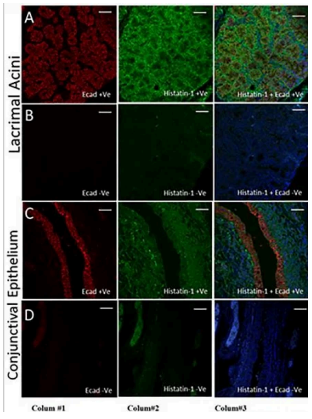
Lane 2:
Western blot - Anti-E-Cadherin/CD324 antibody [M168] - C-terminal (ab76055) at 1/2000 dilution

Lane 3:
Western blot - Anti-E-Cadherin/CD324 antibody [M168] - C-terminal (ab76055) at 1/4000 dilution

All lanes:
A431 (Human epidermoid carcinoma cell line) denatured whole cell lysate at 10 µg

Predicted band size: 97 kDa

Exposure time: 2min



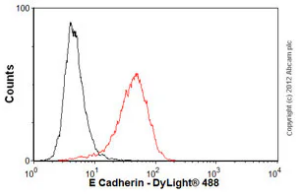
Shah et al PLoS One. 2016 Jan 29;11(1):e0148018. doi: 10.1371/journal.pone.0148018. eCollection 2016. Fig 2. Reproduced under the Creative Commons license

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-E-Cadherin/CD324 antibody [M168] - C-terminal (ab76055)

E-Cadherin/CD324 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-E-Cadherin/CD324 antibody

Immunohistochemistry staining of E Cadherin in human MMCR (Muller's muscle conjunctival resection) tissue.
Xylene and rehydration with serial ethanol dilutions were used for deparaffinization. Slides were washed twice for 5 minutes in 0.25% Triton X-100 for permeabilization and blocked for 2 hours at room temperature with 2% BSA and 10% normal donkey serum in PBS. Slides were incubated overnight at 4°C with ab76055. The next day, the slides were washed twice for 5 minutes in PBS and incubated for 1–2 hours with respective secondary antibodies diluted in blocking solution (1:200–800). Vecta shield mounting medium with 4′,6-diamidino-2-phenylindole (DAPI) was placed over the slides and covered with a glass coverslip. Slides were analyzed using the Zeiss LSM 710 Confocal Microscope.

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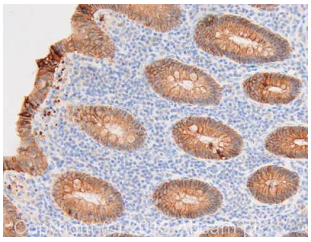
Flow Cytometry (Intracellular) - Anti-E-Cadherin/CD324 antibody [M168] - C-terminal (ab76055)

E-Cadherin/CD324 Flow Cytometry (Intracellular) staining using mouse Anti-E-Cadherin/CD324 antibody

Overlay histogram showing A431 (Human epidermoid carcinoma cell line) cells stained with ab76055 (red line).
The cells were fixed with 4% paraformaldehyde (10 minutes) and then permeabilized with 0.1% PBS-Tween for 20 minutes. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab76055, 1/100 dilution) for 30 minutes at 22°C. The secondary antibody used was a DyLight® 488 goat anti-mouse IgG (H+L) ab96879 at 1/500 dilution for 30 minutes at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2 µg/1x10^6 cells) used under the same conditions.

Acquisition of >5,000 events was performed.

This antibody gave a positive signal in A431 cells fixed with 80% methanol (5 minutes)/permeabilized with 0.1% PBS-Tween for 20 minutes used under the same conditions.

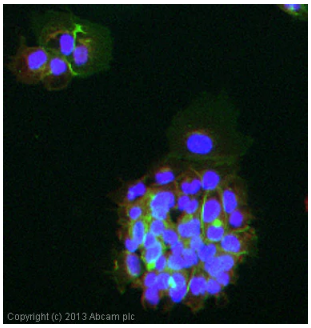


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-E-Cadherin/CD324 antibody [M168] - C-terminal (ab76055)

E-Cadherin/CD324 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using mouse Anti-E-Cadherin/CD324 antibody

IHC image of E Cadherin staining in human colon formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F.
The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer pH 6, for 20 minutes. The section was then incubated with ab76055, 5 µg/ml, for 15 minutes at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunocytochemistry - Anti-E-Cadherin/CD324 antibody [M168] - C-terminal (ab76055)

E-Cadherin/CD324 Immunocytochemistry staining using mouse Anti-E-Cadherin/CD324 antibody

ICC/IF image of ab76055 stained A431 (Human epidermoid carcinoma cell line) cells.
The cells were 4% formaldehyde fixed (10 minutes) and then incubated in 1% BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1 hour to permeabilize the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab76055, 1/100 dilution) overnight at +4°C. The secondary antibody (green) was ab96879, DyLight® 488 goat

anti-mouse IgG (H+L) used at a 1/250 dilution for 1 hour. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1 hour. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43 µM.

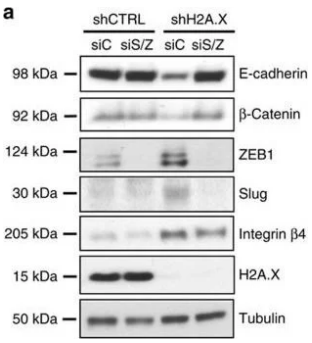


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Western blot - Anti-E-Cadherin/CD324 antibody [M168] - C-terminal (ab76055)

E-Cadherin/CD324 Western blot staining using mouse Anti-E-Cadherin/CD324 antibody

Western Blotting using Anti-E Cadherin antibody [M168] - C-terminal, ab76055. Publication image from Redon, C. E. et al., 2016, Nat Commun, 26876487. Legend direct from paper.

Downregulation of the transcription factors Slug and ZEB1 reverses the EMT programme induced by H2A.X deficiency.(a) Control cells (shCTRL) and cells silenced for H2A.X (shH2A.X) were transfected for 3 days with control siRNA (siC) or with a pool of siSLUG and siZEB1 (siS/Z). Immunoblot analysis of EMT markers was performed using tubulin as loading control. (b) Staining of E-cadherin and β-catenin by immunofluorescence in HCT116 control cells (shCTRL) and H2A.X-deficient cells (shH2A.X) transfected for 3 days with control siRNA (siC) or a pool of siRNAs against SLUG and ZEB1 (siS/Z). Nuclei were counterstained with propidium iodide (red); scale bars, 20 µm. (c) Top panel, analysis of cell phenotypic changes through the staining of F-actin (phalloidin) by immunofluorescence using conditions described in b; scale bars, 20 µm. Bottom panel, photomicrographs of cells; scale bar, 100 µm.(d) Immunoblot analysis of EMT markers in HCT116 parental cells (WT) and H2A.X knockout cells (KO) transfected for 3 days with siRNA control (siC) or with a pool of siSLUG and siZEB1 (siS/Z) with tubulin-loading controls. H2A.X knockout cells were generated using CRISPR/Cas9 system for precise deletion of the H2A.X gene. cl.5, clone #5; and cl.6, clone #6. (e) Immunofluorescence assays showing E-cadherin and β-catenin staining. Cells were treated as in d. Nuclei were counterstained in red with propidium iodide; scale bars, 20 µm.

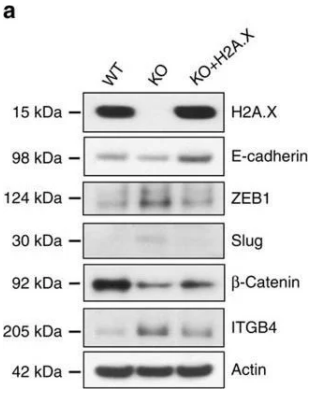


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H2A.X re-expression inhibits EMT and promotes metastatic colonization in the lung.(a) Immunoblot analysis of EMT markers in HCT116 parental cells (WT), H2A.X knockout cells (KO) and H2A.X knockout cells in which H2A.X expression was restored (KO+H2A.X), utilizing actin as a loading control. (b) HCT116 parental cells (WT), H2A.X knockout cells (KO) and revertant cells (KO+H2A.X) were analysed for transcripts levels of EMT markers by real-time PCR. Expression values for E-cadherin (CDH1), ZEB1, Slug, Vimentin (VIM), integrin beta 4 (ITGB4) and versican (VCAN) were normalized to 18S RNA (gene/18S ratio). Error bars represent the s.e. (n=3). Statistical significance was determined by a two-tailed, unpaired Student's t-test. The experiments were repeated three times. (c). Immunostaining of the epithelial marker E-cadherin. Nuclei were counterstained in red with propidium iodide; scale bars, 20 µm. Photomicrographs of cells are shown (top panel); scale bar, 100 µm. (d) Tail vein injections of HCT116 parental cells (WT) and H2A.X knockout cells (KO) showed similar numbers of lung metastatic foci 4 weeks post injection. Ectopic expression of H2A.X (KO+H2A.X) resulted in a 2.5-fold increase in lung macroscopic metastases. (e) Representative haematoxylin- and eosin-stained lung sections from mice injected with HCT116 parental cells (WT), H2A.X knockout cells (KO) and H2A.X knockout cells in which H2A.X expression was restored (KO+H2A.X). Black arrows indicate some macroscopic lung nodules. Scale bars, 1 mm. Statistical significance was determined by a two-tailed, unpaired Student's t-test.

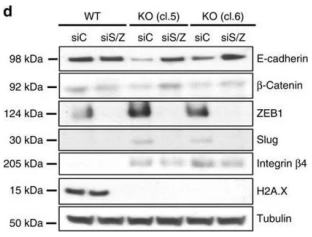


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Western blot - Anti-E-Cadherin/CD324 antibody [M168] - C-terminal (ab76055)

E-Cadherin/CD324 Western blot staining using mouse Anti-E-Cadherin/CD324 antibody

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