abcam

ab256580

Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free

Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580) is a rabbit recombinant monoclonal antibody provided in a PBS only buffer for easy conjugation detecting E-Cadherin/CD324 in **Western Blot, Flow Cytometry (Intra), IHC-P, ICC/IF, mIHC** for **Human**.

- KO validated for confirmed specificity
- BSA, sodium azide, and glycerol-free for easy conjugation
- Multiplex IHC validated on the Leica BOND® MAX using Opal reagents
- Biophysical QC for unrivalled batch-batch consistency

Recombinant | RabMAb | Advanced Validation | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EP700Y
Purification technique	Affinity purification Protein A
Dissociation constant	2.8×10^{-11} M
Concentration	0.973 - 1.044 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

mIHC

Tested

Species	Human
Dilution info	1/3000
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human

Dilution info	-
Notes	-

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 (PubMed:29999492). Has a potent invasive suppressor role. It is a ligand for integrin alpha-E/beta-7. E-Cad/CTF2 promotes non-amyloidogenic degradation of Abeta precursors. Has a strong inhibitory effect on APP C99 and C83 production. (Microbial infection) Serves as a receptor for Listeria monocytogenes; internalin A (InlA) binds 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	+4°C
Storage information	Do Not Freeze

Notes

What is this antibody validated in?
Anti-E Cadherin antibody [EP700Y] - BSA and Azide free (ab256580) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Flow Cytometry (Flow Cyt), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF), Multiplex IHC (mIHC) in Human samples.

What is the molecular weight of E Cadherin?
Anti-E Cadherin [EP700Y] - BSA and Azide free (ab256580) specifically detects a band for E Cadherin (UniProt: P12830) at a molecular weight of 97kDa.

Specificity confirmed
The specificity of Anti-E Cadherin antibody [EP700Y] - BSA and Azide free (ab256580) has been confirmed by Immunocytochemistry/ Immunofluorescence testing in CDH1 Knockout A431 cell line.

Other related products
We have a range of other formats of antibody clone [EP700Y] also available for your convenience: ab40772, Alexa Fluor® 488 - ab185013, Alexa Fluor® 647 - ab194982, Carrier free - ab201499, Alexa Fluor® 555 - ab206878, Alexa Fluor® 594 - ab206880, PE - ab224959, APC - ab224960, Carrier free - ab256580

Species reactivity
Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

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

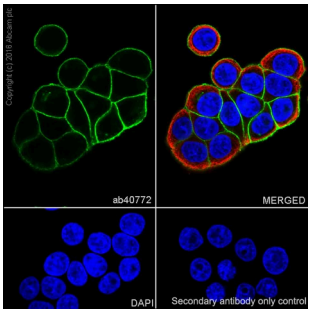
We do not recommend this combination. It is not covered by our product promise.

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:

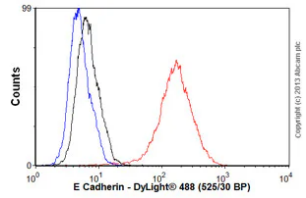
24 product images



Immunocytochemistry/ Immunofluorescence - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

E-Cadherin/CD324 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-E-Cadherin/CD324 antibody

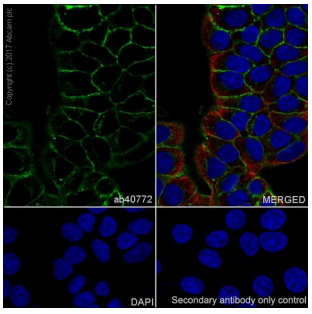
ab40772 staining E Cadherin in HT-29 (Human colorectal adenocarcinoma) cells by ICC/IF (Immunocytochemistry/Immunofluorescence). Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% tritonX-100. Samples were incubated with primary antibody at 1/500 dilution. An Alexa Fluor® 488 Goat anti-Rabbit (ab150077) was used as the secondary antibody at 1/1000 dilution. Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) at 1/200 dilution was used as a counterstain. DAPI was used as a nuclear counterstain. This is a confocal image showing membranous staining on HT-29 cell line.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40772).



Flow Cytometry (Intracellular) - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

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

Overlay histogram showing A431 (Human epidermoid carcinoma cell line) cells stained with unpurified ab40772 (red line). The cells were fixed with 80% methanol (5 minutes) and incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab40772, 1/1000 dilution) for 30 minute at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 minutes at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1 µg/1x10^6 cells) used under the same conditions. Unlabeled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40772).



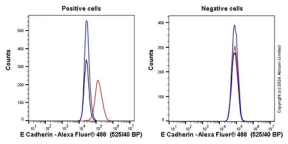
Immunocytochemistry/ Immunofluorescence - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

E-Cadherin/CD324 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-E-Cadherin/CD324 antibody

Immunocytochemistry/Immunofluorescence analysis of MCF7 (human breast adenocarcinoma epithelial) cells labeling E Cadherin with ab40772. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% tritonX-100. Samples were then incubated with the primary antibody at a 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at a 1/1000 dilution (green). The nuclear counter stain is DAPI (blue). Counterstained with ab195889 anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at a 1/200 dilution (red).

Confocal image shows membranous staining on MCF7 cell line.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40772).



Flow Cytometry (Intracellular) - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

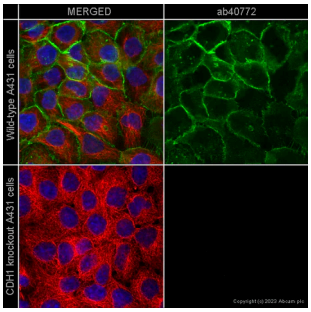
E-Cadherin/CD324 Flow Cytometry (Intracellular) staining using rabbit Anti-E-Cadherin/CD324 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40772).
Flow cytometry overlay histogram showing MCF7 positive cells (left) and negative MDA-MB-231 cells (right) stained with ab40772 (red line). The cells were incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction, followed by the antibody ab40772 (1x 10^6 in 100µl at 5 µg/ml (1/400)) for 30min on ice.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min on ice

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

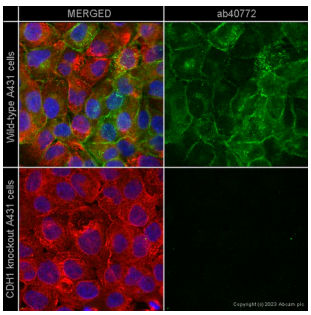
Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Immunocytochemistry/ Immunofluorescence - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

E-Cadherin/CD324 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-E-Cadherin/CD324 antibody

Immunofluorescence staining of E-Cadherin using ab40772 in wild-type A431 cells (top panel) and CDH1 knockout A431 cells (bottom panel). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton-X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab40772 at 0.2 µg/mL and ab7291 at 1 µg/mL overnight at +4°C, followed by a further incubation at room temperature for 1h with Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) (shown in green) and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (ab150120) (shown in red), both at 1/1000. Nuclear DNA was labelled with DAPI (shown in blue). Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a confocal section is shown. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40772).

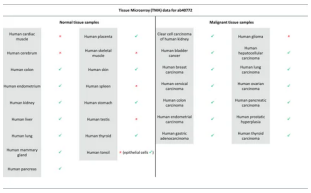


Immunocytochemistry/ Immunofluorescence - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

E-Cadherin/CD324 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-E-Cadherin/CD324 antibody

Immunofluorescence staining of E-Cadherin using ab40772 in wild-type A431 cells (top panel) and CDH1 knockout A431 cells (bottom panel). The cells were fixed with 4% formaldehyde (10 min),

permeabilized with 0.1% Triton-X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab40772 at 1 µg/mL and ab7291 at 1 µg/mL overnight at +4°C, followed by a further incubation at room temperature for 1h with Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) (shown in green) and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (ab150120) (shown in red), both at 1/1000. Nuclear DNA was labelled with DAPI (shown in blue). Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a confocal section is shown. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40772).

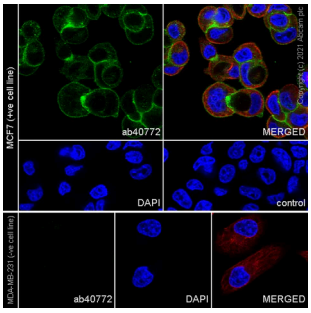


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40772).
Tissue Microarrays stained for Anti-E Cadherin antibody [EP700Y] - Intercellular Junction Marker using ab40772 in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negative (cross mark) staining per sample type tested. The section was incubated with ab40772 for 30 mins at room temperature followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution 2) for 20 mins.



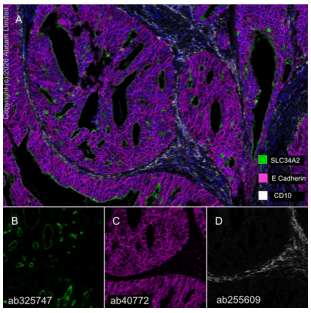
Immunocytochemistry/ Immunofluorescence - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

E-Cadherin/CD324 Immunocytochemistry/ Immunofluorescence staining of CF7 (Human breast adenocarcinoma cell line) whole cell lysate using rabbit Anti-E-Cadherin/CD324 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40772).
Benchmark: ab40772 (recombinant rabbit monoclonal antibody). The data in this figure was generated using ab40772. This antibody was used as a reference for comparison with ab287970. The nuclear counterstain is DAPI (blue).

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized MCF7 (Human breast adenocarcinoma cell line) whole cell lysate labeling E Cadherin with ab40772 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150081) secondary antibody at 1/1000 dilution (green). Confocal image showing membranous staining in MCF7 cell line. Tubulin is detected with Anti-alpha Tubulin mouse MAb (ab195889) at 1/200 dilution (red).

Negative control: MDA-MB-231 (PMID: 10545506).



Multiplex immunohistochemistry - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

E-Cadherin/CD324 Multiplex immunohistochemistry staining of Human endometrial cancer using rabbit Anti-E-Cadherin/CD324 antibody

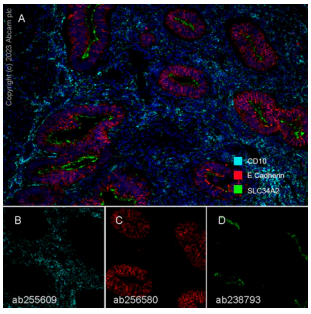
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40772).
Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded human endometrial cancer tissue staining SLC34A2 with ab325747 at a 1:500 (0.99 ug/ml) dilution, ab40772 anti-E Cadherin used at 1:1000 (0.325 ug/ml) dilution and ab255609 anti-CD10 used at a 1:1000 (0.615 ug/ml) dilution.

Panel A: merged staining of anti-SLC34A2 (green; Opal™520), anti-E Cadherin (magenta; Opal™570) and anti-CD10 (gray; Opal™690) on human endometrial cancer.
Panel B: anti-SLC34A2 staining apical cell membrane of glandular cells in human endometrial cancer.
Panel C: anti-E Cadherin staining glandular cells in human endometrial cancer.
Panel D: anti-CD10 staining stromal cells in human endometrial cancer.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab325747, ab40772 and ab255609 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Multiplex immunohistochemistry - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

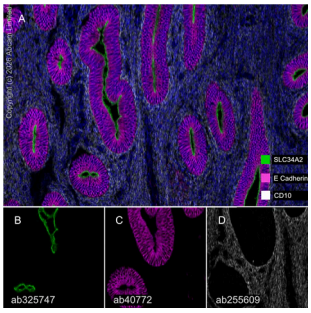
E-Cadherin/CD324 Multiplex immunohistochemistry staining of Human endometrium tissue using rabbit Anti-E-Cadherin/CD324 antibody

Fluorescence multiplex immunohistochemical analysis of the human endometrium (Formalin/PFA-fixed paraffin-embedded sections).
Panel A: merged staining of anti-E Cadherin (ab256580, red; Opal™690), anti-SLC34A2 (ab238793, green; Opal™520) and anti-CD10 (ab255609, cyan; Opal™570) on human endometrium. Panel B: anti-CD10 stained on stromal cells. Panel C: anti-E Cadherin stained on glandular cells. Panel D: anti-SLC34A2 stained on apical membrane of glandular cells. Opal Polymer HRP Ms + Rb was used as a secondary antibody.

The section was incubated in three rounds of staining: in the order of ab256580 at 1/3000 dilution (0.324 µg/ml) for 30 mins, ab238793 at 1/1000 dilution (2.26 µg/ml) for 10 mins and ab255609 at 1/1000 dilution (0.615 µg/ml) for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins. DAPI (blue) was used as a nuclear counterstain.



Multiplex immunohistochemistry - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

E-Cadherin/CD324 Multiplex immunohistochemistry staining of Human endometrium using rabbit Anti-E-Cadherin/CD324 antibody

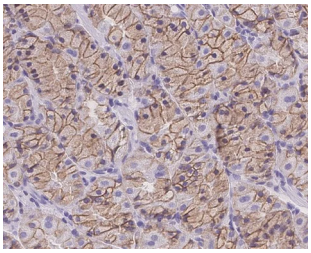
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40772).
Multiplex immunohistochemistry analysis of formalin/PFA-fixed paraffin-embedded human endometrium tissue staining SLC34A2 with ab325747 at a 1:500 (0.99 ug/ml) dilution, ab40772 anti-E Cadherin used at 1:1000 (0.325 ug/ml) dilution and ab255609 anti-CD10 used at a 1:1000 (0.615 ug/ml) dilution.

Panel A: merged staining of anti-SLC34A2 (green; Opal™520), anti-E Cadherin (magenta; Opal™570) and anti-CD10 (gray; Opal™690) on human endometrium.
Panel B: anti-SLC34A2 staining apical cell membrane of glandular cells in human endometrium.
Panel C: anti-E Cadherin staining glandular cells in human endometrium.
Panel D: anti-CD10 staining stromal cells in human endometrium.
Nuclear DNA was labeled with DAPI (shown in blue).

The section was incubated in three rounds of staining: in the order of ab325747, ab40772 and ab255609 for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. Image acquisition was performed with Leica SP8 confocal microscope.

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

E-Cadherin/CD324 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human Stomach using rabbit Anti-E-Cadherin/CD324 antibody

This data was developed using ab40772, the same antibody clone in a different buffer formulation.
Immunohistochemical analysis of paraffin-embedded Human stomach tissue labeling E Cadherin with ab40772 at 1/14000 dilution.

Positive staining on human stomach.

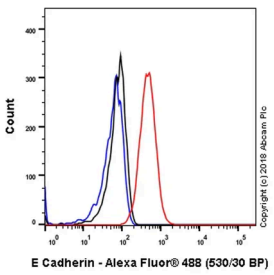
The section was incubated with ab40772 for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary detection reagent (ready-to-use).

Counterstained with Hematoxylin.

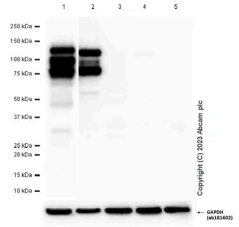
Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Flow Cytometry (Intracellular) - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

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

Intracellular Flow Cytometry analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling E Cadherin with purified ab40772 at 1/30 dilution (10µg/ml) (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40772).



Western blot - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40772). Blocking and diluting buffer and concentration: 5% NFDM/TBST. ab181602 was as GAPDH loading control. Exposure time: Lane1: 3 seconds; Lane 2-5: 40 seconds. A375, HeLa and HT-1080 were reported as negative or express low level of E cadherin (PMID: 30393081, PMID: 16980628, PMID: 34715746), PMID: 25411788).
All lanes:
Western blot - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker (ab40772) at 1/1000 dilution

Lane 1:
MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysate at 20 µg

Lane 3:
A375 (Human malignant melanoma epithelial cell) whole cell lysate at 20 µg

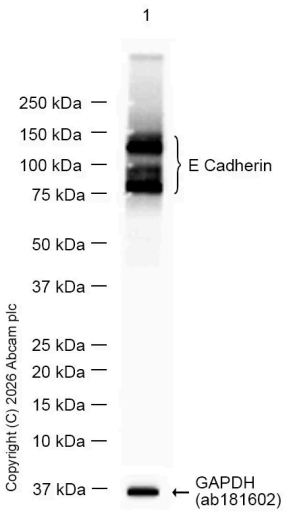
Lane 4:
HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 5:
HT-1080 (Human fibrosarcoma epithelial cell) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 97 kDa



Western blot - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

E-Cadherin/CD324 Western blot staining of U266B1 (human multiple myeloma B lymphocyte) whole cell lysate using rabbit Anti-E-Cadherin/CD324 antibody

This data was developed using ab40772, the same antibody clone in a different buffer formulation. Blocking and diluting buffer and concentration: 5% NFDM/TBST.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/1000,000 dilution.

The full-length of E-cadherin is 120 kDa. The other bands are due to proteolytic cleavages in different Cadherin domains. (PMID: 14695147)

All lanes:
Western blot - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker (ab40772) at 1/1000 dilution

All lanes:
U266B1 (human multiple myeloma B lymphocyte) whole cell lysate at 20 µg

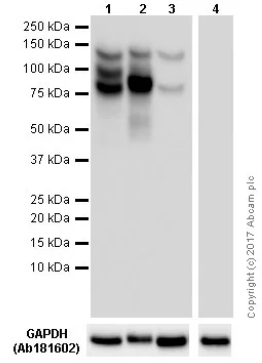
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 120 kDa

Observed band size: 80-120 kDa, 36 kDa

Exposure time: 7s



Western blot - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40772).
Blocking and diluting buffer: 5% NFDM/TBST.

Multi-bands can refer to PMID: 11212238; PMID: 14695147 and PMID: 22659456

All lanes:
Western blot - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker (ab40772) at 1/10000 dilution

Lane 1:
MCF7 (Human breast adenocarcinoma epithelial cell). Whole cell lysates at 20 µg

Lane 2:
HT-29 (Human colorectal adenocarcinoma epithelial cell). Whole cell lysates at 20 µg

Lane 3:
PC-3 (Human prostate adenocarcinoma epithelial cell) Whole cell lysates at 20 µg

Lane 4:
MDA-MB-231 (Human breast adenocarcinoma epithelial cell) Whole cell lysates (negative control) at 20 µg

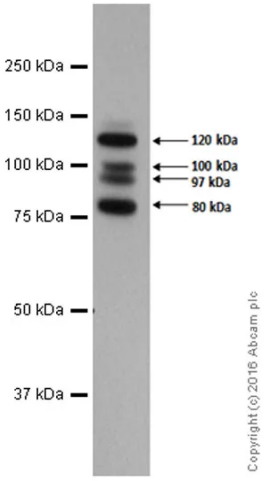
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Performed under reducing conditions.

Predicted band size: 97 kDa

Exposure time: 23s



Western blot - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

E-Cadherin/CD324 Western blot staining of MCF7 (Human breast adenocarcinoma) whole cell lysates using rabbit Anti-E-Cadherin/CD324 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40772).
Blocking and diluting buffer and concentration 5% NFDM/TBST.
The full-length of E-cadherin is 120 kDa. The other bands are due to proteolytic cleavages in different Cadherin domains. (Ref: PMID: 14695147)

All lanes:
Western blot - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker (ab40772) at 1/200000 dilution

All lanes:
MCF7 (Human breast adenocarcinoma) whole cell lysates at 20 µg

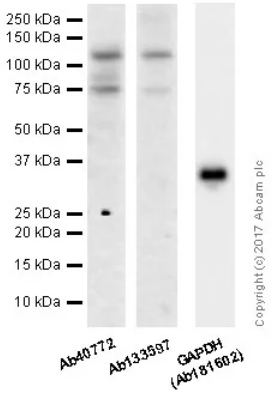
Secondary

All lanes:
Goat Anti-Rabbit IgG H&L (HRP) at 1/1000 dilution

Predicted band size: 97 kDa

Observed band size: 100 kDa, 120 kDa, 80 kDa, 97 kDa

Exposure time: 1min



Western blot - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

E-Cadherin/CD324 Western blot staining of PC-3 (Human prostate adenocarcinoma epithelial cell) Whole cell lysates using rabbit Anti-E-Cadherin/CD324 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40772).
Exposure time: 3 minutes for ab40772 and ab133597, 32 seconds for GAPDH.

Blocking and diluting buffer: 5% NFDM/TBST

Multi-bands can refer to PMID: 11212238; PMID: 14695147 and PMID: 22659456

Lane 1:
Western blot - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker (ab40772) at 1/5000 dilution

Lane 2:
Western blot - Anti-E Cadherin antibody [EPR699] (ab133597) at 1/2000 dilution

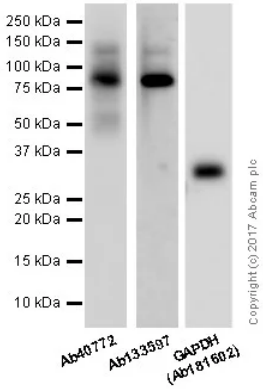
Lane 3:
Western blot - Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602)

All lanes:
PC-3 (Human prostate adenocarcinoma epithelial cell) Whole cell lysates at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 97 kDa



Western blot - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

E-Cadherin/CD324 Western blot staining of HT-29 (Human colorectal adenocarcinoma epithelial cell). Whole cell lysates using rabbit Anti-E-Cadherin/CD324 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40772).
Exposure time: 1 second for ab40772, 3 minutes for ab133597, 32 seconds for GAPDH

Blocking and diluting buffer: 5% NFDM/TBST

Multi-bands can refer to PMID: 11212238; PMID: 14695147 and PMID: 22659456

Lane 1:
Western blot - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker (ab40772) at 1/5000 dilution

Lane 2:
Western blot - Anti-E Cadherin antibody [EPR699] (ab133597) at 1/2000 dilution

Lane 3:
Western blot - Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602)

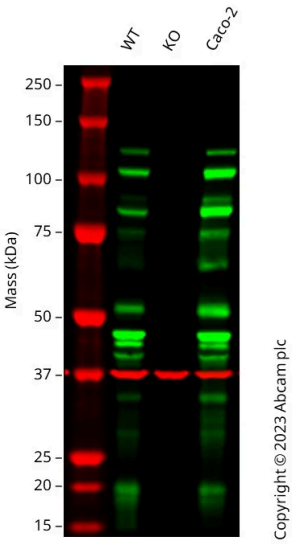
All lanes:
HT-29 (Human colorectal adenocarcinoma epithelial cell). Whole cell lysates at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 97 kDa

Observed band size: 80 kDa



Western blot - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40772). Western blot: Anti-CDH1 antibody [EP700Y] (ab40772) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab40772 was shown to bind specifically to CDH1. A band was observed at 130, 110, 80, 55, 40 kDa in wild-type A431 cell lysates with no signal observed at this size in CDH1 knockout cell line ab273747 (knockout cell lysate ab273781). To generate this image, wild-type and CDH1 knockout A431 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker (ab40772) at 1/1000 dilution

Lane 1:
Wild-type A431 cell lysate at 20 µg

Lane 2:
Western blot - Human CDH1 (E Cadherin) knockout A-431 cell lysate (ab273781)

Lane 2:
Western blot - Human CDH1 (E Cadherin) knockout A-431 cell line (ab273747)

Lane 2:
CDH1 knockout A431 cell lysate at 20 µg

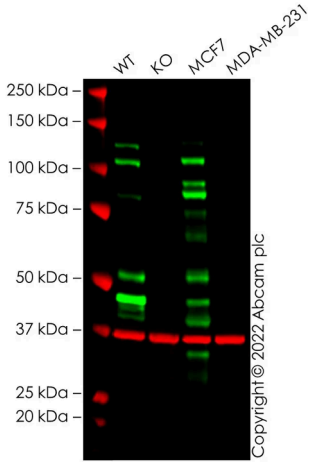
Lane 3:
Caco-2 cell lysate at 20 µg

Secondary

All lanes:
Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 97 kDa



Western blot - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

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

This data was developed using the same antibody clone in a different buffer formulation (ab40772). False colour image of Western blot: Anti-E Cadherin antibody [EP700Y] - Intercellular Junction Marker staining at 1/10000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab40772 was shown to bind specifically to E Cadherin. A band was observed at 105/130 kDa in wild-type Raji cell lysates with no signal observed at this size in CDH1 knockout cell line ab273747 (knockout cell lysate ab273781). To generate this image, wild-type and CDH1 knockout Raji cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose

membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
Lanes 1 - 4:
Western blot - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker (ab40772) at 1/10000 dilution

Lanes 1 - 4:
Western blot - Anti-E-Cadherin/CD324 antibody [EP700Y] - Low endotoxin, Azide free (ab201499) at 1/10000 dilution

Lanes 1 - 4:
Western blot - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580) at 1/10000 dilution

Lane 1:
Wild-type Raji cell lysate at 20 µg

Lane 2:
CDH1 knockout Raji cell lysate at 20 µg

Lane 3:
MCF7 cell lysate at 20 µg

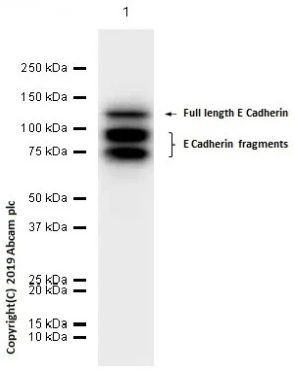
Lane 4:
MDA-MB-231 cell lysate at 20 µg

Secondary

All lanes:
Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Observed band size: 105 kDa, 130 kDa



Western blot - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

E-Cadherin/CD324 Western blot staining of MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate using rabbit Anti-E-Cadherin/CD324 antibody

Exposure time: 3.25 seconds. Blocking and diluting buffer: 5% NFDM/TBST. Full-length E Cadherin has a molecular weight of approximately 125 kDa. Other molecular weights between 80-100 kDa could also be observed depending on cell types or cell conditions. PMID: 27274359, PMID: 26983597, PMID: 18478055, PMID: 22375065. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40772).
All lanes:
Western blot - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker (ab40772) at 1/1000 dilution

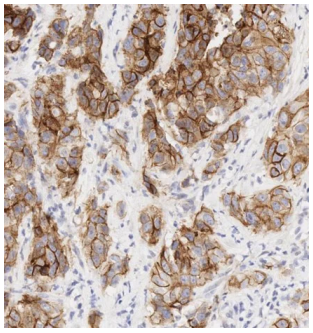
All lanes:
MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 97 kDa

Observed band size: 80-125 kDa

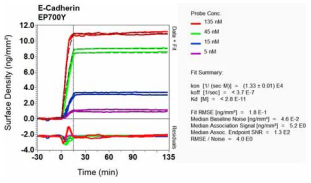


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

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

Immunohistochemistry of breast carcinoma staining E Cadherin with ab40772 at 1µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab40772).



OI-RD Scanning - Anti-E-Cadherin/CD324 antibody [EP700Y] - BSA and Azide free (ab256580)

We have systematically measured KD (the equilibrium dissociation constant between the antibody and its antigen), of more than 840 recombinant antibodies to assess not only their individual KD values but also to see the average affinity of antibody. Based on the comparison with published literature values for mouse monoclonal antibodies, Recombinant antibodies appear to be on average 1-2 order of magnitude higher affinity.

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