

Product datasheet

Anti-E Cadherin antibody [4A2] ab231303

KO VALIDATED

★★★★☆ 2 Abreviews 84 References 8 Images

Overview

Product name	Anti-E Cadherin antibody [4A2]
Description	Mouse monoclonal [4A2] to E Cadherin
Host species	Mouse
Tested applications	Suitable for: WB, ICC/IF, IHC-P
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.
Epitope	The 4A2 monoclonal recognizes the cytoplasmic domain of E-cadherin. The epitope has been mapped to residues 757–778 (PubMed ID: 12393869).
Positive control	ICC/IF: MCF7 and wild-type A431 cells. IHC-P: FFPE human colon carcinoma, rat large intestine and mouse large intestine tissue sections. WB: MCF7 cells, rat and mouse colon tissue lysates.
General notes	<p>This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com.</p> <p>The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.</p> <p>If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.02% Sodium azide Constituent: PBS
Purity	Protein G purified
Clonality	Monoclonal

Clone number	4A2
Isotype	IgG1
Light chain type	kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab231303 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use a concentration of 1 µg/ml. Predicted molecular weight: 97 kDa. Abcam recommends using a 5% milk blocking solution for this antibody.
ICC/IF		Use a concentration of 1 µg/ml.
IHC-P	★★★★★ (1)	Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Target

Function Cadherins are calcium-dependent cell adhesion proteins. 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. 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.

Tissue specificity Non-neural epithelial tissues.

Involvement in disease Defects in CDH1 are the cause of hereditary diffuse gastric cancer (HDGC) [MIM:137215]. An autosomal dominant cancer predisposition syndrome with increased susceptibility to diffuse gastric cancer. Diffuse gastric cancer is a malignant disease characterized by poorly differentiated infiltrating lesions resulting in thickening of the stomach. Malignant tumors start in the stomach, can spread to the esophagus or the small intestine, and can extend through the stomach wall to nearby lymph nodes and organs. It also can metastasize to other parts of the body. Note=Heterozygous germline mutations CDH1 are responsible for familial cases of diffuse gastric cancer. Somatic mutations in the has also been found in patients with sporadic diffuse gastric cancer and lobular breast cancer.
Defects in CDH1 are a cause of susceptibility to endometrial cancer (ENDMC) [MIM:608089]. Defects in CDH1 are a cause of susceptibility to ovarian cancer (OC) [MIM:167000]. Ovarian cancer common malignancy originating from ovarian tissue. Although many histologic types of ovarian neoplasms have been described, epithelial ovarian carcinoma is the most common form. Ovarian cancers are often asymptomatic and the recognized signs and symptoms, even of late-stage disease, are vague. Consequently, most patients are diagnosed with advanced disease.

Sequence similarities Contains 5 cadherin domains.

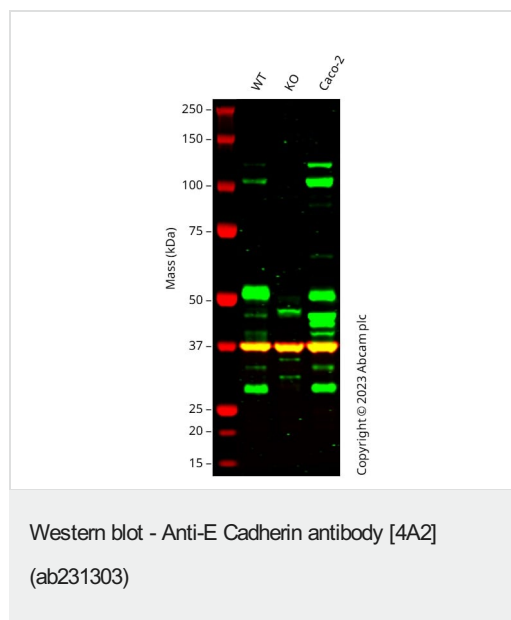
Post-translational modifications

During apoptosis or with calcium influx, cleaved by a membrane-bound metalloproteinase (ADAM10), PS1/gamma-secretase and caspase-3 to produce fragments of about 38 kDa (E-CAD/CTF1), 33 kDa (E-CAD/CTF2) and 29 kDa (E-CAD/CTF3), respectively. Processing by the metalloproteinase, induced by calcium influx, causes disruption of cell-cell adhesion and the subsequent release of beta-catenin into the cytoplasm. The residual membrane-tethered cleavage product is rapidly degraded via an intracellular proteolytic pathway. Cleavage by caspase-3 releases the cytoplasmic tail resulting in disintegration of the actin microfilament system. The gamma-secretase-mediated cleavage promotes disassembly of adherens junctions.

Cellular localization

Cell junction. Cell membrane. Endosome. Golgi apparatus > trans-Golgi network. Colocalizes with DLGAP5 at sites of cell-cell contact in intestinal epithelial cells. Anchored to actin microfilaments through association with alpha-, beta- and gamma-catenin. Sequential proteolysis induced by apoptosis or calcium influx, results in translocation from sites of cell-cell contact to the cytoplasm. Colocalizes with RAB11A endosomes during its transport from the Golgi apparatus to the plasma membrane.

Images



All lanes : Anti-E Cadherin antibody [4A2] (ab231303) at 1 µg/ml

Lane 1 : Wild-type A431 cell lysate

Lane 2 : CDH1 knockout A431 cell lysate

Lane 3 : Caco-2 cell lysate

Lysates/proteins at 20 µg per lane.

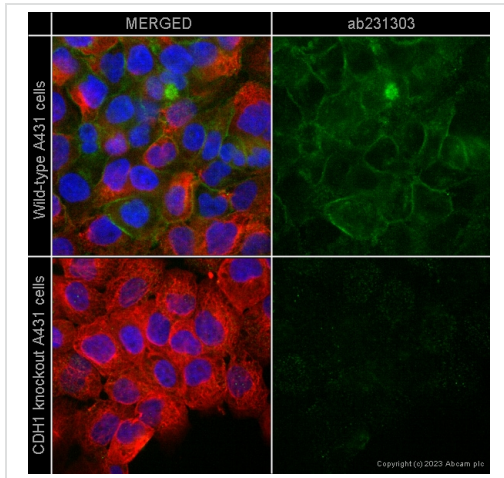
Performed under reducing conditions.

Predicted band size: 97 kDa

Observed band size: 110,130,55 kDa

Western blot: Anti-CDH1 antibody [4A2] (ab231303) staining at 1 µg/ml, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] ([ab181602](#)) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab231303 was shown to bind specifically to CDH1. A band was observed at 130, 110, 55 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 5 % 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

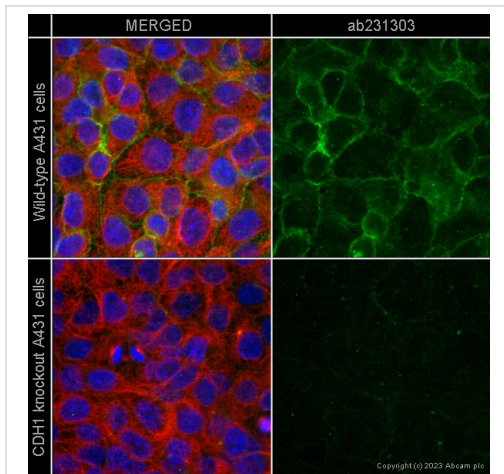
for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Mouse IgG H&L 800CW and Goat anti-Rabbit IgG H&L 680RD at 1/20000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-E Cadherin antibody [4A2] (ab231303)

Immunofluorescence staining of E-Cadherin using ab231303 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 ab231303 at 1.0 µg/mL and **ab6046** at 1 µg/mL overnight at +4°C, followed by a further incubation at room temperature for 1h with Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed (**ab150117**) (shown in green) and Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) preadsorbed (**ab150084**) (shown in red), both at 1/1000. Nuclear DNA was labelled with DAPI (shown in blue).

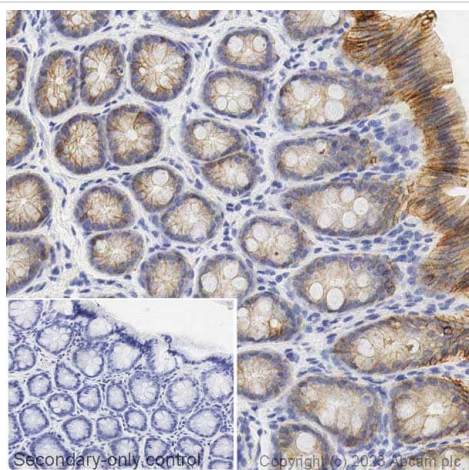
Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a confocal section is shown.



Immunocytochemistry/ Immunofluorescence - Anti-E Cadherin antibody [4A2] (ab231303)

Immunofluorescence staining of E-Cadherin using ab231303 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 ab231303 at 1.0 µg/mL and **ab6046** at 1 µg/mL overnight at +4°C, followed by a further incubation at room temperature for 1h with Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed (**ab150117**) (shown in green) and Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) preadsorbed (**ab150084**) (shown in red), both at 1/1000. Nuclear DNA was labelled with DAPI (shown in blue).

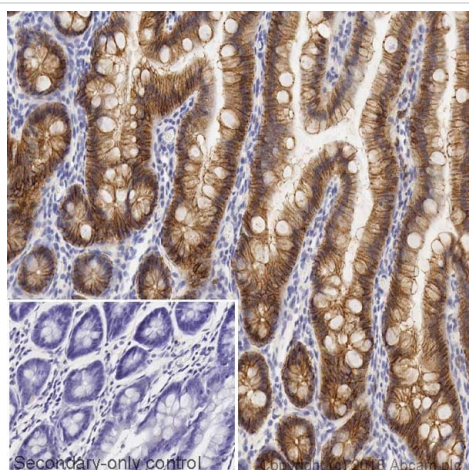
Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a confocal section is shown.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-E Cadherin antibody [4A2] (ab231303)

IHC image of E-Cadherin staining in a section of formalin-fixed paraffin-embedded normal mouse large intestine performed on a Leica Biosystems BOND® RX instrument. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab231303, 1ug/ml, for 15 mins 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. The inset secondary-only control image is taken from an identical assay without primary antibody.

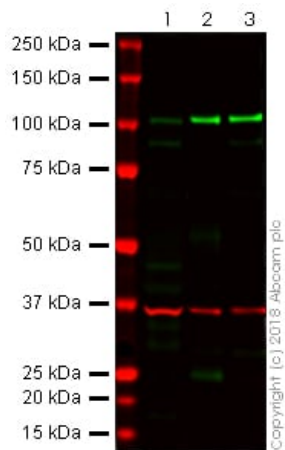
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-E Cadherin antibody [4A2] (ab231303)

IHC image of E Cadherin staining in a section of formalin-fixed paraffin-embedded normal rat large intestine 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 (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab231303, 5ug/ml, for 15 mins 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. The inset secondary-only control image is taken from an identical assay without primary antibody.

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.



Western blot - Anti-E Cadherin antibody [4A2]
(ab231303)

All lanes :

Lane 1 : MCF7 whole cell lysate

Lane 2 : Mouse colon tissue lysate

Lane 3 : Rat colon tissue lysate

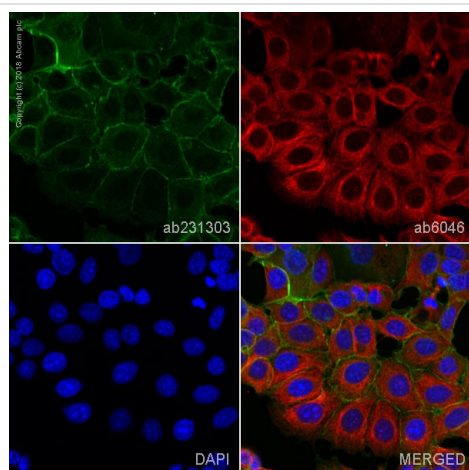
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 97 kDa

Observed band size: 105 kDa

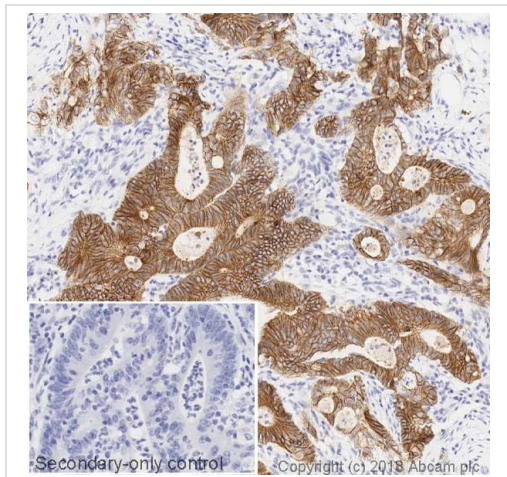
This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 55 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% milk before ab231303 and **ab181602** (Rabbit anti-GAPDH loading control) were incubated overnight at 4°C at a 1µg/ml concentration and 1/10000 dilution respectively. Antibody binding was detected using Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-E
Cadherin antibody [4A2] (ab231303)

ab231303 staining E-Cadherin in MCF7 cells. The cells were fixed with 4% PFA (10min), permeabilized with 0.1% PBS-Tween 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 overnight at +4°C with ab231303 at 1µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control, at 1/1000 dilution. Cells were then incubated with **ab150117**, Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) at 1/1000 dilution (shown in green) and **ab150084**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolor red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-E Cadherin antibody [4A2] (ab231303)

IHC image of E Cadherin staining in a section of formalin-fixed paraffin-embedded normal human colon carcinoma* 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 (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab231303, 1ug/ml, for 15 mins 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. The inset secondary-only control image is taken from an identical assay without primary antibody.

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.

**Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre*

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