Overview

Product name  Anti-E Cadherin antibody [EP700Y] - Intercellular Junction Marker
Description  Rabbit monoclonal [EP700Y] to E Cadherin - Intercellular Junction Marker
Host species  Rabbit
Specificity  E-cadherin contains a number of cleavage sites which may yield a complex fragmentation pattern in WB. Multiple bands between ~80-120 kDa may be observed. This antibody has been tested on human samples in both WB and IHC. Customer feedback (see Abreview) suggests the antibody does not perform well in IHC on mouse tissue.

Tested applications  Suitable for: IHC-Fr, ICC/IF, IHC-P, Flow Cyt, WB
Species reactivity  Reacts with: Human
Immunogen  Synthetic peptide within Human E Cadherin aa 600-700. The exact sequence is proprietary. Database link: P12830

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

Abcam recommended secondaries - Goat Anti-Rabbit HRP (ab205718) and Goat Anti-Rabbit Alexa Fluor® 488 (ab150077).

See other anti-rabbit secondary antibodies that can be used with this antibody.

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

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.
This product is a recombinant rabbit monoclonal antibody.

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. Avoid freeze / thaw cycle.

**Dissociation constant ($K_D$)**  
$K_D = 2.80 \times 10^{-11}$ M

**Storage buffer**  
pH: 7.20  
Preservative: 0.01% Sodium azide  
Constituents: PBS, 40% Glycerol, 0.5% BSA

**Purity**  
Protein A purified

**Clonality**  
Monoclonal

**Clone number**  
EP700Y

**Isotype**  
IgG

Applications

Our Abpromise guarantee covers the use of ab40772 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC-Fr</td>
<td>Use at an assay dependent concentration. PubMed: 24915897</td>
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<tr>
<td>ICC/IF</td>
<td>1/500. Permeabilisation is unnecessary as the immunogen is in an extracellular domain.</td>
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<tr>
<td>IHC-P</td>
<td>1/500. See IHC antigen retrieval protocols.</td>
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</tbody>
</table>
| Flow Cyt    | 1/30.  
ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. For purified format use at 1/1000. |
| WB          | 1/10000 - 1/50000. Detects a band of approximately 80-120 kDa (predicted molecular weight: 97 kDa). |

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**
**Western blot** - Anti-E Cadherin antibody [EP700Y] - Intercellular Junction Marker (ab40772)

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

- **Lane 1**: MCF7 (Human breast adenocarcinoma epithelial cell). Whole cell lysates

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

- **Lane 3**: PC-3 (Human prostate adenocarcinoma epithelial cell) Whole cell lysates

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

Lysates/proteins at 20 µg per lane.

**Secondary**

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

Performed under reducing conditions.

**Predicted band size**: 97 kDa

**Exposure time**: 23 seconds

Blocking and diluting buffer: 5% NFDM/TBST

Multi-bands can refer to PMID: 11212238; PMID: 14695147 and PMID: 22659456
PMA induced cell fusion, DYSF expression, and activation of PKC in BeWo cells while 4αPMA was inactive

Immunofluorescence analysis of BeWo cells treated with 0.25% DMSO (controls), 10 nM PMA, or 10 nM 4αPMA for 72 h. The cells were then fixed and subsequently double-labeled for detection of DYSF (red) and E-cadherin (green). Nuclei were labeled with DAPI. While there can be a low level of spontaneous fusion in control cells (in our hands this ranges from about 4 to 9%), most cells are not fused and have at their borders intact E-cadherin labeling. Moreover, DYSF labeling was not detectable in non-fused BeWo cells. However, treatment of BeWo cells with 10 nM PMA for 72 h led to increased levels of cell fusion as indicated by the breakdown of E-cadherin labeling and the expression of DYSF in fused cells. When BeWo cells were treated with 10 nM 4αPMA for 72 h there was no detectable increase in cell fusion or DYSF expression. Arrows indicate areas enlarged and placed in insets. Bar = 50 μm.

Mesenchymal cancer cells show increased metastasis while not requiring MET for solid tumor formation.

ZEB1 or E-cadherin staining of metastases in ICI-mice. Note the higher E-cad and lower ZEB1 expression in the metastatic cells expressing OVOL1 or ZEB1-shRNA (sh4). Scale bar represents 100 μm.
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.

Flow Cytometry analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling E Cadherin with purified ab40772 at 1:30 dilution (10 µg/ml) (red). 10⁶ 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).
Fluorescent immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using unpurified ab40772. Green-E-Cadherin red-PI.

Overlay histogram showing A431 (Human epidermoid carcinoma cell line) cells stained with unpurified ab40772 (red line). 10^6 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.
Formalin/PFA-fixed paraffin-embedded human colonic adenocarcinoma tissue stained for E Cadherin with unpurified ab40772 at a 1/500 dilution in immunohistochemical analysis.

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

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

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

**All lanes**: PC-3 (Human prostate adenocarcinoma epithelial cell)

Whole cell lysates

Lysates/proteins at 20 µg per lane.

**Secondary**

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

**Predicted band size**: 97 kDa

**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
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.

Formalin-fixed, paraffin-embedded human lung adenocarcinoma tissue stained for E Cadherin with unpurified ab40772 at a 1/500 dilution in immunohistochemical analysis.
Lane 1: Anti-E Cadherin antibody [EP700Y] - Intercellular Junction Marker (ab40772) at 1/5000 dilution

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

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

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

Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 97 kDa

Observed band size: 80 kDa

why is the actual band size different from the predicted?

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
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-E Cadherin antibody [EP700Y] - Intercellular Junction Marker (ab40772)

Formalin-fixed, paraffin-embedded human breast carcinoma tissue stained for E Cadherin with unpurified ab40772 at a 1/500 dilution in immunohistochemical analysis.

Western blot - Anti-E Cadherin antibody [EP700Y] - Intercellular Junction Marker (ab40772)

Anti-E Cadherin antibody [EP700Y] - Intercellular Junction Marker (ab40772) at 1/200000 dilution (Unpurified) + MCF7 (Human breast adenocarcinoma) whole cell lysates at 20 µg

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

**Predicted band size:** 97 kDa
**Observed band size:** 100,120,80,97 kDa why is the actual band size different from the predicted?

**Exposure time:** 1 minute

 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)
Formalin-fixed, paraffin-embedded human papillary carcinoma of thyroid gland tissue stained for E Cadherin with unpurified ab40772 at a 1/500 dilution in immunohistochemical analysis.

Formalin-fixed, paraffin-embedded human transitional cell carcinoma of kidney tissue stained for E Cadherin with unpurified ab40772 at a 1/500 dilution in immunohistochemical analysis.
Immunohistochemistry of breast carcinoma staining E Cadherin with ab40772 at 1μg/ml

Produced using unpurified ab40772
Equilibrium disassociation constant (K_D)
Learn more about K_D
Click here to learn more about K_D

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