

Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker

Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker (ab40772) is a rabbit monoclonal antibody detecting E-Cadherin/CD324 in **Western Blot, Flow Cytometry (Intra), IHC-P, ICC/IF, mIHC**. Suitable for **Human**.

- KO validated for confirmed specificity
- Multiplex IHC validated on the Leica BOND® MAX using Opal reagents
- Biophysical QC for unrivalled batch-batch consistency
- Over 750 publications

Lab Essentials

Recombinant

RabMAb

Advanced Validation

KO Validated

20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EP700Y
Purification technique	Affinity purification Protein A
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.
Dissociation constant	2.8 x 10 ⁻¹¹ M
Concentration	0.326 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

mIHC

Tested

Species	Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/500 - 1/14000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

WB

Tested

Species	Human
Dilution info	1/1000 - 1/50000
Notes	For unpurified protein: 1/200000 dilution

ICC/IF

Tested

Species	Human
Dilution info	0.2-1 µg/mL
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/30
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. For purified format use at 1/1000.

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.
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Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
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

Abcam recommended secondaries - Goat Anti-Rabbit HRP (ab205718) and Goat Anti-Rabbit Alexa Fluor® 488 (ab150077). Or search our wide range of secondary antibodies for use with your experiment.

Product Specifications

Anti-E Cadherin antibody [EP700Y] - Intercellular Junction Marker (ab40772) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt, Flow Cyt (Intra), ICC/IF, IHC-P, WB, mIHC in human samples.

Anti-E Cadherin antibody [EP700Y] - Intercellular Junction Marker (ab40772) specifically detects E Cadherin (UniProt ID: P12830; Molecular weight: 80kDa) and is sold in a convenient trial size to enable initial testing (20 µL) and larger sizes for subsequent scaling up experiments (100 µL and 1 mL).

Quality and Validation

Abcam's high quality manufacturing and validation processes ensure Anti-E Cadherin antibody [EP700Y] - Intercellular Junction Marker (ab40772) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.

The specificity of Anti-E Cadherin antibody [EP700Y] - Intercellular Junction Marker (ab40772) has been confirmed by testing in knockout samples.

Anti-E Cadherin antibody [EP700Y] - Intercellular Junction Marker (ab40772) has been cited over 750 times in peer reviewed journals and is trusted by the scientific community.

Anti-E Cadherin antibody [EP700Y] - Intercellular Junction Marker (ab40772) has 21 independent reviews from customers.

Related Products

Antibody clone EP700Y is also available pre-conjugated to a variety of labels for your convenience - Alexa Fluor® 488, Alexa Fluor® 647, Alexa Fluor® 555, Alexa Fluor® 594, PE, APC (ab185013, ab194982, ab206878, ab206880, ab224959, ab224960).

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.

Species reactivity

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

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 β -catenin a significant player in transcription regulation and cell signaling. Disruption in E-Cadherin's adhesive functionality can lead to increased β -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 β -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

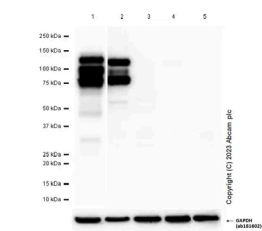
We do not recommend this combination. It is not covered by our product promise.

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.

26 product images



Western blot - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker (ab40772)

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

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

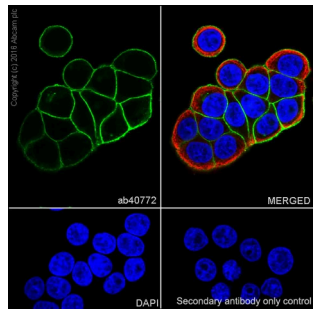
Lane 4:
HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

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

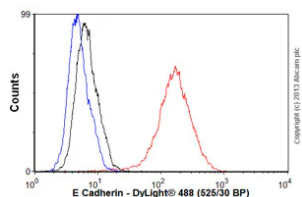


Immunocytochemistry/ Immunofluorescence - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker (ab40772)

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

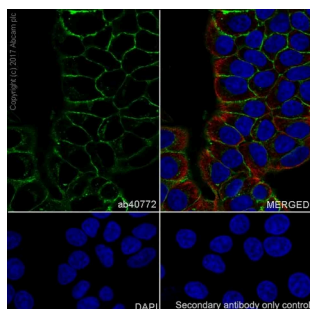
dilution. An Alexa Fluor® 488 Goat anti-Rabbit ([ab150077](#)) was used as the secondary antibody at 1/1000 dilution. Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) ([ab195889](#)) at 1/200 dilution was used as a counterstain. DAPI was used as a nuclear counterstain. This is a confocal image showing membranous staining on HT-29 cell line.



Flow Cytometry (Intracellular) - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker ([ab40772](#))

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). 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) (0.1µg/1x10⁶ 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.

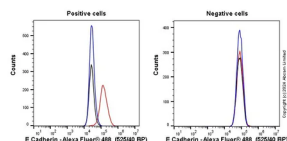


Immunocytochemistry/ Immunofluorescence - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker ([ab40772](#))

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.



Flow Cytometry (Intracellular) - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker ([ab40772](#))

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

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 10⁶ 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.

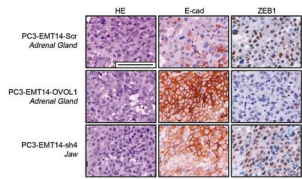


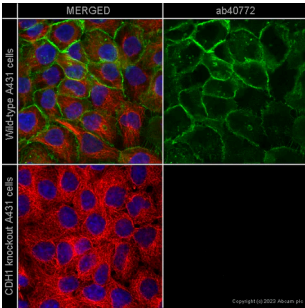
Image from Roca H. et al
PLoS One. 2013 Oct
4;8(10):e76773. doi:
10.1371/journal.pone.007
6773. eCollection 2013.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker (ab40772)

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

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

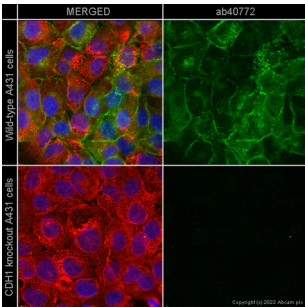
ZEB1 or E-cadherin staining of metastases in ICI-mice. Note the higher E-cad and lower ZEB1 expression in the metastatic cells expressing OV01 or ZEB1-shRNA (sh4). Scale bar represents 100 μ m.



Immunocytochemistry/ Immunofluorescence - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker (ab40772)

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

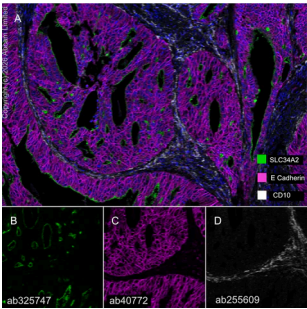
Immunofluorescence staining of E-Cadherin using ab40772 in wild-type A431 cells (top panel) and CDH1 knockout A431 cells (bottom panel). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton-X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab40772 at 0.2 μ g/mL and [ab7291](#) at 1 μ g/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.



Immunocytochemistry/ Immunofluorescence - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker (ab40772)

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 μ g/mL and [ab7291](#) at 1 μ g/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.



Multiplex immunohistochemistry - Anti-E-Cadherin/CD324 antibody [EP700Y] - InterCellular Junction Marker (ab40772)

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

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.

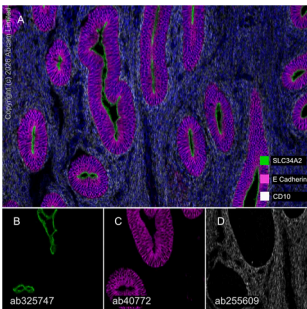
Tissue Microarrays (TMA) data for ab40772			
Formalin-fixed paraffin-embedded (FFPE) sections		Immunohistochemistry (IHC) results	
Sample ID	Staining Result	Sample ID	Staining Result
1	Positive	1	Positive
2	Positive	2	Positive
3	Positive	3	Positive
4	Positive	4	Positive
5	Positive	5	Positive
6	Positive	6	Positive
7	Positive	7	Positive
8	Positive	8	Positive
9	Positive	9	Positive
10	Positive	10	Positive
11	Positive	11	Positive
12	Positive	12	Positive
13	Positive	13	Positive
14	Positive	14	Positive
15	Positive	15	Positive
16	Positive	16	Positive
17	Positive	17	Positive
18	Positive	18	Positive
19	Positive	19	Positive
20	Positive	20	Positive

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-E-Cadherin/CD324 antibody [EP700Y] - InterCellular Junction Marker (ab40772)

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

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.



Multiplex immunohistochemistry - Anti-E-Cadherin/CD324 antibody [EP700Y] - InterCellular Junction Marker (ab40772)

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

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.

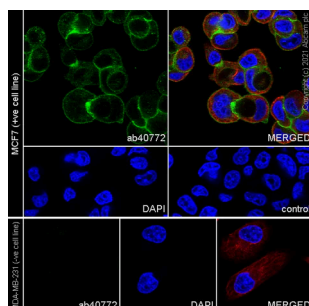
Panel D: anti-CD10 staining stromal cells in human endometrium.

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.



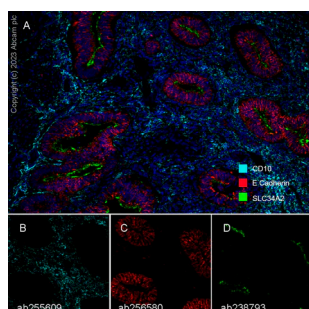
Immunocytochemistry/ Immunofluorescence - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker (ab40772)

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

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] - Intercellular Junction Marker (ab40772)

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.

This data was developed using the same antibody clone in a different buffer formulation ([ab256580](#)).

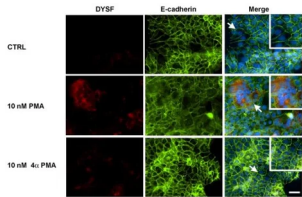


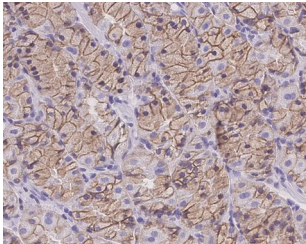
Image from Omata W. et al
PLoS One. 2013 Nov
13;8(11):e81003. doi:
10.1371/journal.pone.008
1003. eCollection 2013.

Immunocytochemistry/ Immunofluorescence - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker (ab40772)

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

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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker (ab40772)

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

Immunohistochemical analysis of paraffin-embedded Human stomach tissue labeling E Cadherin with ab40772 at 1/14000 dilution.

Positive staining on human stomach.

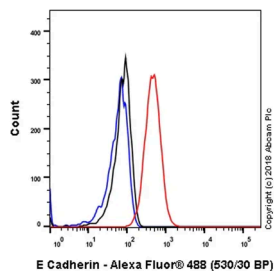
The section was incubated with ab40772 for 30 mins at room temperature.

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.

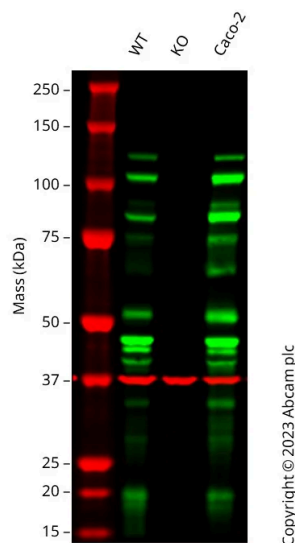
Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Flow Cytometry (Intracellular) - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker (ab40772)

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). 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).



Western blot - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker (ab40772)

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

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

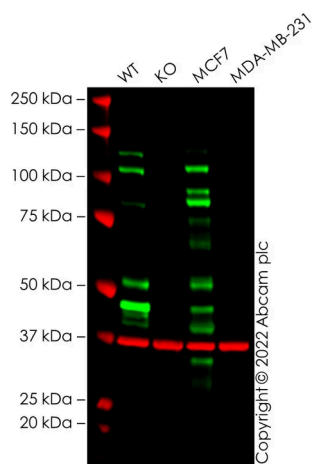
Secondary

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] - Intercellular Junction Marker (ab40772)

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

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

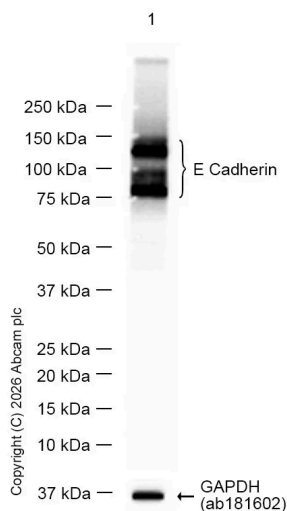
Secondary

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] - Intercellular Junction Marker (ab40772)

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

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

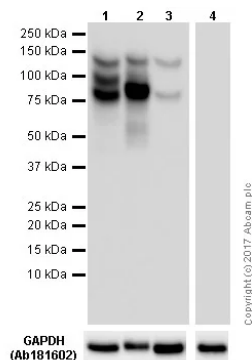
All lanes:

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] - Intercellular Junction Marker (ab40772)

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

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

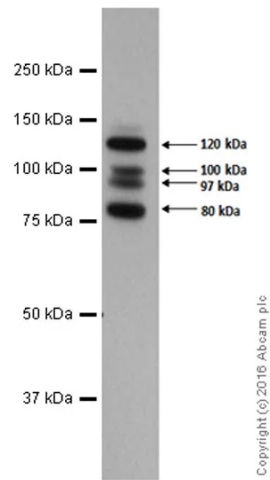
All lanes:

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] - Intercellular Junction Marker (ab40772)

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

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

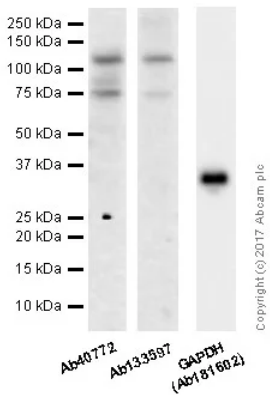
All lanes:

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] - Intercellular Junction Marker (ab40772)

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

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:

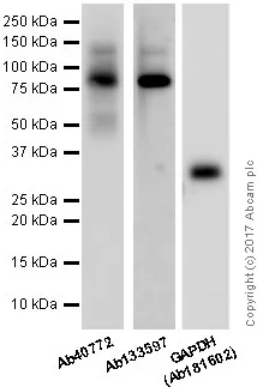
Western blot - Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602)

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] - Intercellular Junction Marker (ab40772)

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

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](#))

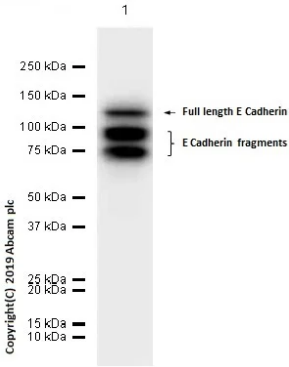
All lanes:
HT-29 (Human colorectal adenocarcinoma epithelial cell). Whole cell lysates at 20 µg

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] - Intercellular Junction Marker (ab40772)

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.

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

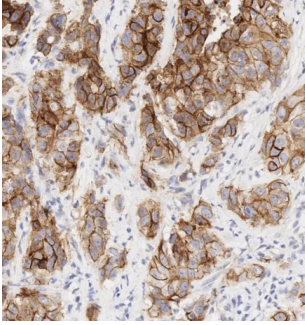
All lanes:
MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Secondary

Western blot - Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

Predicted band size: 97 kDa

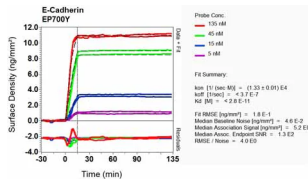
Observed band size: 80-125 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker (ab40772)

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.



OI-RD Scanning - Anti-E-Cadherin/CD324 antibody [EP700Y] - Intercellular Junction Marker (ab40772)

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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