abcam

Product datasheet

Anti-ADAM10 antibody [EPR5622] ab124695





★★★★★ 4 Abreviews 34 References 8 Images

Overview

Product name Anti-ADAM10 antibody [EPR5622]

Description Rabbit monoclonal [EPR5622] to ADAM10

Host species Rabbit

Tested applications Suitable for: WB, IP

Unsuitable for: IHC-P

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide within Human ADAM10 (C terminal). The exact sequence is proprietary.

Positive control Jurkat, RAW 264.7, U2OS, LnCaP and MCF-7 cell lysates.

General notes 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 production

For more information see here.

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

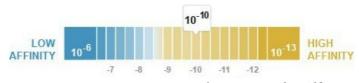
Properties

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. Stable for 12 months at -20°C.

 $K_D = 1.80 \times 10^{-10} M$ Dissociation constant (K_D)



Learn more about K_D

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol (glycerin, glycerine), 0.05% BSA, 59% PBS

Purity Protein A purified

Clonality Monoclonal
Clone number EPR5622

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab124695 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	★★★★ <u>(2)</u>	1/1000 - 1/10000. Predicted molecular weight: 84 kDa.	
IP		1/10 - 1/100.	

Application notes Is unsuitable for IHC-P.

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Function Cleaves the membrane-bound precursor of TNF-alpha at '76-Ala-

-Val-77' to its mature soluble form. Responsible for the proteolytical release of soluble JAM3 from endothelial cells surface. Responsible for the proteolytic release of several other cell-surface proteins, including heparin-binding epidermal growth-like factor, ephrin-A2 and for constitutive and regulated alpha-secretase cleavage of amyloid precursor protein (APP). Contributes to the normal cleavage of the cellular prion protein. Involved in the cleavage of the adhesion molecule L1 at the cell surface and in released membrane vesicles, suggesting a vesicle-based protease activity. Controls also the proteolytic processing of Notch and mediates lateral inhibition during

Tissue specificity Expressed in spleen, lymph node, thymus, peripheral blood leukocyte, bone marrow, cartilage,

chondrocytes and fetal liver.

Sequence similarities Contains 1 disintegrin domain.

Contains 1 peptidase M12B domain.

Domain The conserved cysteine present in the cysteine-switch motif binds the catalytic zinc ion, thus

inhibiting the enzyme. The dissociation of the cysteine from the zinc ion upon the activation-

peptide release activates the enzyme.

Post-translational

modifications

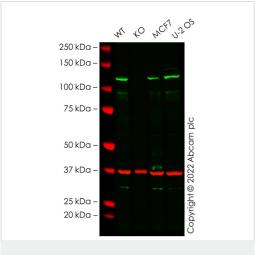
The precursor is cleaved by a furin endopeptidase.

Cellular localization Cell membrane. Endomembrane system. Is localized in the plasma membrane but is

predominantly expressed in the Golgi apparatus and in released membrane vesicles derived

likely from the Golgi.

Images



Western blot - Anti-ADAM10 antibody [EPR5622] (ab124695)

All lanes : Anti-ADAM10 antibody [EPR5622] (ab124695) at 1/1000 dilution

Lane 1: Wild-type Jurkat cell lysate at 10 µg

Lane 2: ADAM10 knockout Jurkat cell lysate at 10 µg

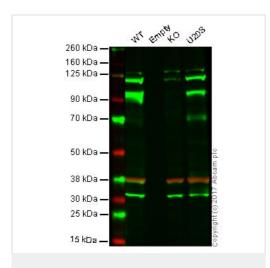
Lane 3: MCF7 cell lysate at 20 μg **Lane 4**: U-2 OS cell lysate at 20 μg

Performed under reducing conditions.

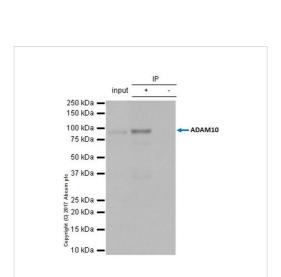
Predicted band size: 84 kDa

Observed band size: 95-120 kDa

False colour image of Western blot: Anti-ADAM10 antibody [EPR5622] 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, ab124695 was shown to bind specifically to ADAM10. A band was observed at 95/120 kDa in wild-type Jurkat cell lysates with no signal observed at this size in ADAM10 knockout cell line. The band at 120 kDa is likely to be the precursor and the band at 90 kDa is likely to be the active form of ADAM10. To generate this image, wild-type and ADAM10 knockout Jurkat 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.



Western blot - Anti-ADAM10 antibody [EPR5622] (ab124695)



Immunoprecipitation - Anti-ADAM10 antibody [EPR5622] (ab124695)

Lane 1: Wild type HAP1 whole cell lysate (40 µg)

Lane 2: Empty lane

Lane 3: ADAM10 knockout HAP1 whole cell lysate (40 µg)

Lane 4: U20S whole cell lysate (40 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab124695 observed at 90 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

ab124695 was shown to recognize ADAM10 when ADAM10 knockout samples were used, along with additional cross-reactive bands. Wild-type and ADAM10 knockout samples were subjected to SDS-PAGE. Ab124695 and ab8245 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

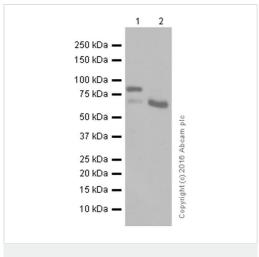
ab124695 (purified) at 1:30 dilution (2ug) immunoprecipitating ADAM-10 in LNCaP (Human prostate carcinoma epithelial cell) whole cell lysate 10ug.

Lane 1 (input): LNCaP (Human prostate carcinoma epithelial cell) whole cell lysate 10ug

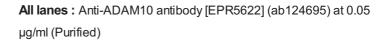
Lane 2 (+): ab124695 & LNCaP (Human prostate carcinoma epithelial cell) whole cell lysate 10ug

Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) instead of <u>ab170952</u> in HeLa LNCaP (Human prostate carcinoma epithelial cell) whole cell lysateFor western blotting, VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) was used for detection at 1:1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.



Western blot - Anti-ADAM10 antibody [EPR5622] (ab124695)



Lane 1 : RAW264.7 (Mouse Abelson murine leukemia virusinduced tumor macrophage) whole cell lysate

Lane 2: Rat brain lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 84 kDa



Western blot - Anti-ADAM10 antibody [EPR5622] (ab124695)

Blocking and diluting buffer: 5% NFDM/TBST

All lanes : Anti-ADAM10 antibody [EPR5622] (ab124695) at 0.09 μg/ml (Purified)

Lane 1 : Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate

Lane 2 : MCF-7 (Human breast adenocarcinoma epithelial cell) whole cell lysate

Lane 3 : LNCaP (Human prostate carcinoma epithelial cell) whole cell lysate

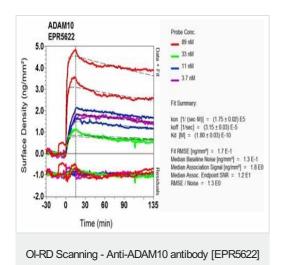
Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 84 kDa

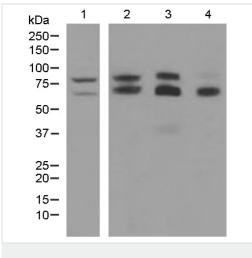
Blocking and diluting buffer: 5% NFDM/TBST



(ab124695)

Equilibrium disassociation constant (K_D) Learn more about K_D

Click here to learn more about K_D



Western blot - Anti-ADAM10 antibody [EPR5622] (ab124695)

All lanes : Anti-ADAM10 antibody [EPR5622] (ab124695) at 1/1000 dilution

Lane 1: Jurkat cell lysate

Lane 2: RAW 264.7 cell lysate

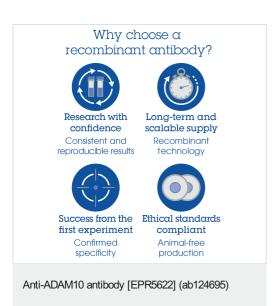
Lane 3 : LnCaP cell lysate

Lane 4 : MCF-7 cell lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 84 kDa

84 kDa band is pro-ADAM10, smaller band at around 65 kDa is mature ADAM10 protein.



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