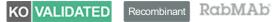
abcam

Product datasheet

Anti-ACE2 antibody [EPR4435(2)] ab108252





★★★★★ 9 Abreviews 121 References 13 Images

Overview

Product name Anti-ACE2 antibody [EPR4435(2)]

Description Rabbit monoclonal [EPR4435(2)] to ACE2

Host species Rabbit

Tested applications Suitable for: WB, IP, IHC-P, Indirect ELISA

Unsuitable for: Flow Cyt or ICC/IF

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

(Peptide available as ab198988)

Positive control WB: Human testis, kidney and lung tissue lysates; Human fetal kidney tissue lysate; Calu-3,

> HepG2 and Caco-2 cell lysates. Human and rat heart tissue lysate; Human lung tissue lysate; Mouse and rat spleen, testis lung tissue lysate; IHC-P: Human, mouse, and rat kidney tissues. IP:

Human testis tissue lysate.

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.

Storage buffer pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA

Purity Protein A purified

Clonality Monoclonal
Clone number EPR4435(2)

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab108252 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 | ★★★★☆ (5) | 1/1000 - 1/10000. Predicted molecular weight: 92 kDa.Can be blocked with ACE2 peptide (ab198988) . |
| IP | | 1/10 - 1/100. |
| IHC-P | **** <u>(1)</u> | 1/6400 - 1/32000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. For unpurified use at 1/100 - 1/250. |
| Indirect ELISA | | Use at an assay dependent concentration. |

Application notes

Is unsuitable for Flow Cyt or ICC/IF.

Target

Function Carboxypeptidase which converts angiotensin I to angiotensin 1-9, a peptide of unknown function,

and angiotensin II to angiotensin 1-7, a vasodilator. Also able to hydrolyze apelin-13 and dynorphin-13 with high efficiency. May be an important regulator of heart function. In case of human coronaviruses SARS and HCoV-NL63 infections, serve as functional receptor for the

spike glycoprotein of both coronaviruses.

Tissue specificity Expressed in endothelial cells from small and large arteries, and in arterial smooth muscle cells.

Expressed in lung alveolar epithelial cells, enterocytes of the small intestine, Leydig cells and Sertoli cells (at protein level). Expressed in heart, kidney, testis, and gastrointestinal system.

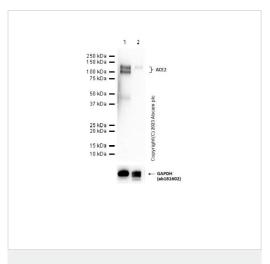
Sequence similarities Belongs to the peptidase M2 family.

Post-translational modifications

N-glycosylation on Asn-90 may limit SARS infectivity.

Cellular localization Secreted and Cell membrane.

Images



Western blot - Anti-ACE2 antibody [EPR4435(2)] (ab108252)

All lanes : Anti-ACE2 antibody [EPR4435(2)] (ab108252) at 1/1000 dilution

Lane 1: Human heart tissue lysate

Lane 2: Rat heart tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Anti-Rabbit lgG (HRP) with minimal cross-reactivity with

human IgG at 1/2000 dilution

Predicted band size: 92 kDa

Observed band size: 110,120 kDa

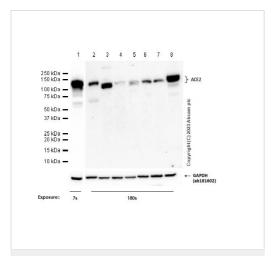
Exposure time: 180 seconds

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

ab181602 was used as a GAPDH loading control.

Two bands observed by ab108252 corresponding to glycosylation and non-glycosylation forms.

Signal in heart tissue is low, we recommend loading more amount of lysate or using lower antibody dilution to improve result.



Western blot - Anti-ACE2 antibody [EPR4435(2)] (ab108252)

All lanes : Anti-ACE2 antibody [EPR4435(2)] (ab108252) at 1/1000 dilution

Lane 1: Human testis tissue lysate at 20 µg

Lane 2: Human lung tissue lysate at 20 µg

Lane 3: Mouse testis tissue lysate

Lane 4: Mouse spleen tissue lysate

Lane 5: Mouse lung tissue lysate

Lane 6: Rat testis tissue lysate

Lane 7: Rat spleen tissue lysate

Lane 8: Rat lung tissue lysate

Secondary

All lanes : Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Predicted band size: 92 kDa

Observed band size: 110,120 kDa

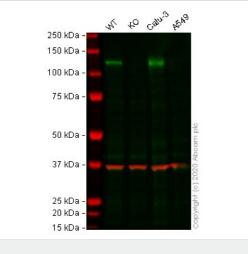
Blocking and diluting buffer and concentration: 5% NFDM/TBST.

ab181602 was used as GAPDH loading control.

Exposure time: Lane 1: 7 seconds; Lane 2-8: 180 seconds.

Two bands observed by ab108252 corresponding to glycosylation and non-glycosylation forms.

Signal in mouse and rat tissues are low, we recommend loading more amount of lysate or using lower antibody dilution to improve result.



Western blot - Anti-ACE2 antibody [EPR4435(2)] (ab108252)

All lanes : Anti-ACE2 antibody [EPR4435(2)] (ab108252) at 1/1000 dilution

Lane 1: Wild-type HepG2 cell lysate

Lane 2: ACE2 knockout HepG2 cell lysate

Lane 3 : Calu-3 cell lysate

Lane 4 : A549 cell lysate

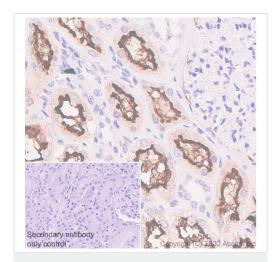
Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Predicted band size: 92 kDa **Observed band size:** 130 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab108252 observed at 130 kDa. Red - loading control <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab108252 was shown to react with ACE2 in wild-type HepG2 cells in western blot with loss of signal observed in ACE2 knockout cell line ab273733 (knockout cell lysate ab275495). Wild-type and ACE2 knockout HepG2 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween[®]) before incubation with ab108252 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated 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 in 20000 dilution for 1 hour at room temperature before imaging.

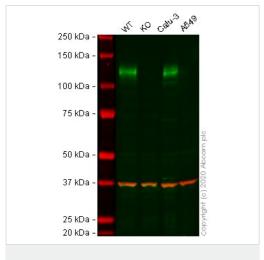


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ACE2 antibody
[EPR4435(2)] (ab108252)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney tissue labeling ACE2 with ab108252 at 1/6400 dilution. Heat mediated antigen retrieval was performed using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes. Staining was visualised using Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

The section was incubated with ab108252 for 30 mins at room temperature.

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



Western blot - Anti-ACE2 antibody [EPR4435(2)] (ab108252)

All lanes : Anti-ACE2 antibody [EPR4435(2)] (ab108252) at 1/1000 dilution

Lane 1: Wild-type Caco-2 cell lysate

Lane 2: ACE2 knockout Caco-2 cell lysate

Lane 3 : Calu-3 cell lysate
Lane 4 : A549 cell lysate

Lysates/proteins at 30 µg per lane.

Performed under reducing conditions.

Predicted band size: 92 kDa **Observed band size:** 125 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab108252 observed at 125 kDa. Red - loading control <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab108252 was shown to react with ACE2 in Caco-2 wild-type cells in western blot with loss of signal observed in ACE2 knockout cell line ab273731 (knockout cell lysate ab275516). Wild-type and ACE2 knockout Caco-2 cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution before incubation with ab108252 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were

incubated with Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) preabsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

1 2 3 4 5 6

250 kDa —

150 kDa —

100 kDa —

75 kDa —

37 kDa —

25 kDa —

20 kDa —

15 kDa —

15 kDa —

Western blot - Anti-ACE2 antibody [EPR4435(2)] (ab108252)

All lanes : Anti-ACE2 antibody [EPR4435(2)] (ab108252) at 1/1000 dilution

Lane 1: Human testis cell lysate

Lane 2: Human kidney cell lysate

Lane 3: Human lung cell lysate

Lane 4: HepG2 cell lysate

Lane 5: Caco-2 cell lysate

Lane 6: A549 cell lysate (negative control)

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 92 kDa

Observed band size: 120 kDa

Lanes 1 - 6: Merged signal (red and green). Green - ab108252 observed at 120 kDa. Red - loading control, Mouse anti-Actin observed at 42kDa.

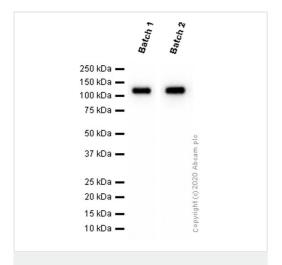
ab108252 was shown to react with ACE2 in western blot.

Membranes were blocked in 3% milk in TBS-T (0.1% Tween[®])

before incubation with ab108252 and Mouse anti Actin overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively.

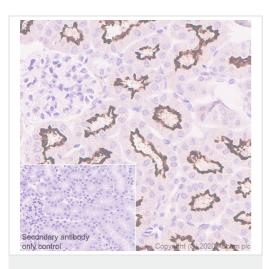
Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Absence of ACE2 expression in A549 cells aligns with previously reported mRNA and protein data (PMID 16282461; fig.2b and 2c).



Different batches of ab108252 were tested on Human kidney lysate at 0.2 μ g/ml. 15 μ g of lysate was loaded in each lane. Bands observed at 120 kDa.



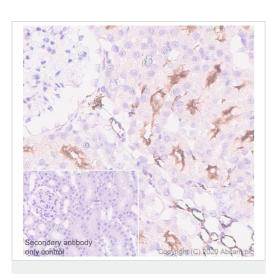


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ACE2 antibody
[EPR4435(2)] (ab108252)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse kidney tissue labeling ACE2 with ab108252 at 1/6400 dilution. Heat mediated antigen retrieval was performed using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes. Staining was visualised using Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

The section was incubated with ab108252 for 30 mins at room temperature.

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

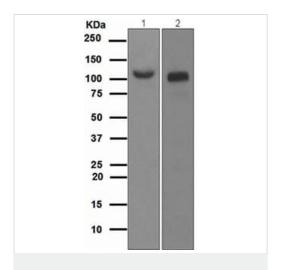


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ACE2 antibody
[EPR4435(2)] (ab108252)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat kidney tissue labeling ACE2 with ab108252 at 1/6400 dilution. Heat mediated antigen retrieval was performed using Bond™ Epitope Retrieval Solution 2 (pH 9.0) for 20 minutes. Staining was visualised using Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

The section was incubated with ab108252 for 30 mins at room temperature.

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



Western blot - Anti-ACE2 antibody [EPR4435(2)] (ab108252)

All lanes : Anti-ACE2 antibody [EPR4435(2)] (ab108252) at 1/1000 dilution

Lane 1: Human fetal kidney lysate

Lane 2: Human testis lysate

Lysates/proteins at 10 µg per lane.

Predicted band size: 92 kDa



[EPR4435(2)] (ab108252)

ab108252 Immunoprecipitating ACE2 in human testis tissue lysate. 0.35 mg of tissue lysate was incubated with 0.6 µg primary antibody (1/20). For western blotting a HRP-conjugated Veriblot for IP Detection Reagent (ab131366) (1/1000) was used to confirm successful immunoprecipitation.

Exposure time: 1 second.

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

All lanes : Anti-ACE2 antibody [EPR4435(2)] (ab108252) at 1/500 dilution

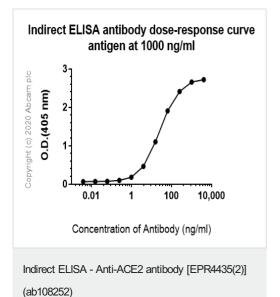
Lane 1: Human testis tissue lysate at 10 µg

Lane 2: ab108252 + Human testis tissue lysate

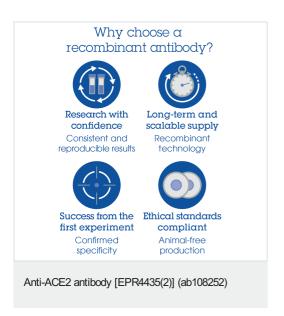
Lane 3 : Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab108252

in Human testis tissue lysate

Observed band size: 110 kDa



ELISA using ab108252 at varying antibody concentrations (4000~0 ng/ml) and antigen concentration at 1000 ng/mL. An Alkaline Phosphatase-conjugated Goat Anti-Rabbit lgG (H+L) (1/2500) was used as the secondary antibody.



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors