abcam

Product datasheet

Anti-Angiotensin Converting Enzyme 1 antibody [EPR22291-247] ab254222

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Angiotensin Converting Enzyme 1 antibody [EPR22291-247]
Description	Rabbit monoclonal [EPR22291-247] to Angiotensin Converting Enzyme 1
Host species	Rabbit
Tested applications	**Suitable for:** IHC-Fr, WB, IHC-P, mIHC, Indirect ELISA **Unsuitable for:** Flow Cyt,ICC/IF or IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: bEND.3, HUVEC and HAP1 whole cell lysates; human kidney and lung cell lysates; mouse brain, heart, kidney, spleen and lung tissue lysates; rat brain, heart, liver and spleen tisuue lysates. IHC-P: Human kidney and liver tissue; mouse kidney tissue; rat kidney tissue. IHC-Fr: Mouse kidney tissue; rat kidney tissue. mIHC-P: Human kidney tissue.
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: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR22291-247
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab254222 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
IHC-Fr		1/500. Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).
WB	★★★★★ (1)	1/1000. Detects a band of approximately 180 kDa (predicted molecular weight: 150 kDa).
IHC-P		1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
mIHC		1/4000.
Indirect ELISA		Use at an assay dependent concentration.

Application notes Is unsuitable for Flow Cyt,ICC/IF or IP.

Target

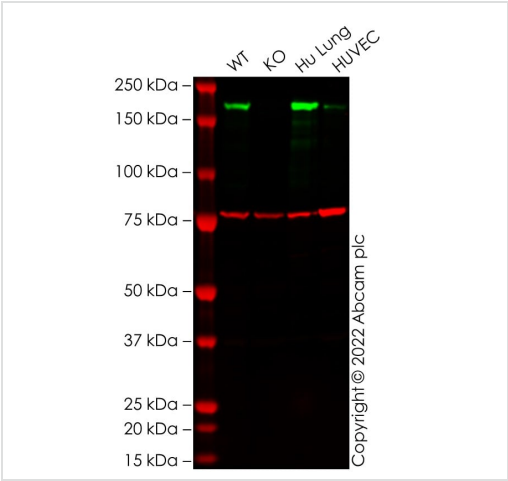
Function Converts angiotensin I to angiotensin II by release of the terminal His-Leu, this results in an increase of the vasoconstrictor activity of angiotensin. Also able to inactivate bradykinin, a potent vasodilator. Has also a glycosidase activity which releases GPI-anchored proteins from the membrane by cleaving the mannose linkage in the GPI moiety.

Tissue specificity Ubiquitously expressed, with highest levels in lung, kidney, heart, gastrointestinal system and prostate. Isoform Testis-specific is expressed in spermatocytes and adult testis.

Involvement in disease Ischemic stroke (ISCHSTR) [MIM:601367]: A stroke is an acute neurologic event leading to death of neural tissue of the brain and resulting in loss of motor, sensory and/or cognitive function. Ischemic strokes, resulting from vascular occlusion, is considered to be a highly complex disease consisting of a group of heterogeneous disorders with multiple genetic and environmental risk factors. Note=Disease susceptibility is associated with variations affecting the gene represented in this entry.
Renal tubular dysgenesis (RTD) [MIM:267430]: Autosomal recessive severe disorder of renal tubular development characterized by persistent fetal anuria and perinatal death, probably due to pulmonary hypoplasia from early-onset oligohydramnios (the Potter phenotype). Note=The disease is caused by mutations affecting the gene represented in this entry.
Microvascular complications of diabetes 3 (MVCD3) [MIM:612624]: Pathological conditions that develop in numerous tissues and organs as a consequence of diabetes mellitus. They include diabetic retinopathy, diabetic nephropathy leading to end-stage renal disease, and diabetic

	neuropathy. Diabetic retinopathy remains the major cause of new-onset blindness among diabetic adults. It is characterized by vascular permeability and increased tissue ischemia and angiogenesis. Note=Disease susceptibility is associated with variations affecting the gene represented in this entry. Intracerebral hemorrhage (ICH) [MIM:614519]: A pathological condition characterized by bleeding into one or both cerebral hemispheres including the basal ganglia and the cerebral cortex. It is often associated with hypertension and craniocerebral trauma. Intracerebral bleeding is a common cause of stroke. Note=Disease susceptibility is associated with variations affecting the gene represented in this entry.
Sequence similarities	Belongs to the peptidase M2 family.
Post-translational modifications	Phosphorylated by CK2 on Ser-1299; which allows membrane retention.
Cellular localization	Secreted and Cell membrane.

Images



Western blot - Anti-Angiotensin Converting Enzyme 1 antibody [EPR22291-247] (ab254222)

All lanes : Anti-Angiotensin Converting Enzyme 1 antibody [EPR22291-247] (ab254222) at 1/1000 dilution

Lane 1 : Wild-type SKNFI cell lysate
Lane 2 : Ace knockout SKNFI cell lysate
Lane 3 : Human Lung cell lysate
Lane 4 : HUVEC cell lysate

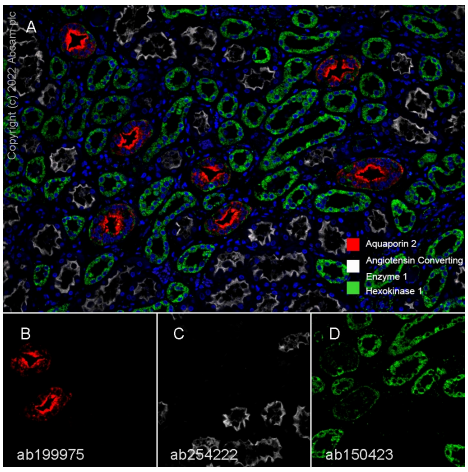
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 150 kDa
Observed band size: 200 kDa

False colour image of Western blot: Anti-Angiotensin Converting Enzyme 1 antibody [EPR22291-247] staining at 1/1000 dilution, shown in green; Mouse anti-CANX [CANX/1543] (**ab238078**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab254222 was shown to bind specifically to Angiotensin Converting Enzyme 1. A band was observed at 200 kDa in wild-type SKNFI cell lysates with no signal observed at this size in Ace knockout cell line **ab288707**. To generate this image, wild-type and Ace knockout SKNFI 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.



Multiplex immunohistochemistry - Anti-Angiotensin Converting Enzyme 1 antibody [EPR22291-247] (ab254222)

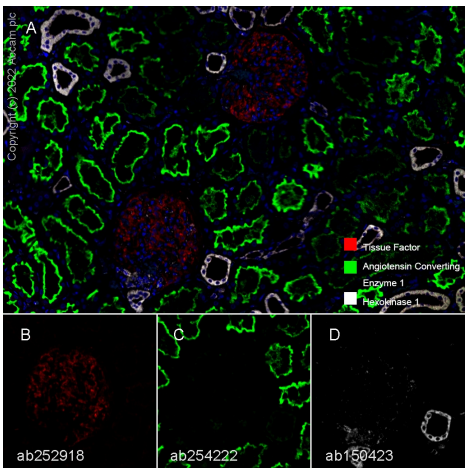
Fluorescence multiplex immunohistochemical analysis of the human kidney (Formalin/PFA-fixed paraffin-embedded sections).

Panel A: merged staining of anti-Hexokinase 1 (**ab150423**, green; Opal™690), anti-Angiotensin Converting Enzyme 1 (ab254222, gray; Opal™520) and anti-Aquaporin 2 (**ab199975**, red; Opal™570) on human kidney. Panel B: anti-Aquaporin 2 stained on collecting tubules. Panel C: anti-Angiotensin Converting Enzyme 1 stained on proximal tubules. Panel D: anti-Hexokinase 1 stained on distal tubules and collecting tubules. Opal Polymer HRP Ms + Rb was used as a secondary antibody.

The section was incubated in three rounds of staining: in the order of **ab150423** at 1/250 dilution (4.224 µg/ml), ab254222 at 1/4000 dilution (0.141 µg/ml) and **ab199975** at 1/4000 dilution (0.152 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 solution2) for 20 mins. DAPI (blue) was used as a nuclear counter stain.



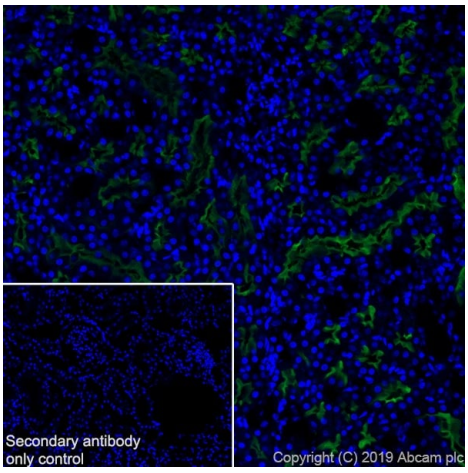
Multiplex immunohistochemistry - Anti-Angiotensin Converting Enzyme 1 antibody [EPR22291-247] (ab254222)

Fluorescence multiplex immunohistochemical analysis of paraffin-embedded Human kidney tissue.

Panel A: Merged staining of anti-Hexokinase 1 (gray; Opal™690), anti-Angiotensin Converting Enzyme 1 (green; Opal™520) and anti-Tissue Factor (red; Opal™570) on human kidney.
Panel B: Anti-Tissue Factor stained on renal glomeruli.
Panel C: Anti-Angiotensin Converting Enzyme 1 stained on proximal tubules.
Panel D: Anti-Hexokinase 1 stained on distal tubules.

The section was incubated in three rounds of staining: in the order of **ab150423**, ab254222, and **ab252918** 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. Counterstained with DAPI.

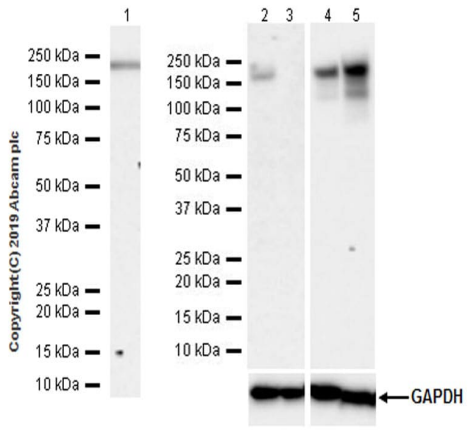


Immunohistochemistry (Frozen sections) - Anti-Angiotensin Converting Enzyme 1 antibody [EPR22291-247] (ab254222)

Immunohistochemical analysis of frozen section of 4% PFA-fixed, 0.2% Triton X-100 permeabilized rat kidney tissue labeling Angiotensin Converting Enzyme 1 with ab254222 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution (green). Positive staining on proximal tubules of kidney (PMID:10504496) is observed. The nuclear counter stain is DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution.

Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).



Western blot - Anti-Angiotensin Converting Enzyme 1 antibody [EPR22291-247] (ab254222)

All lanes : Anti-Angiotensin Converting Enzyme 1 antibody [EPR22291-247] (ab254222) at 1/1000 dilution

Lane 1 : HUVEC (human umbilical vein endothelial cell line) whole cell lysate at 20 µg
Lane 2 : Wild-type HAP1 whole cell lysate at 40 µg
Lane 3 : Angiotensin Converting Enzyme 1 knockout HAP1 whole cell lysate at 40 µg
Lane 4 : Human kidney cell lysate at 40 µg
Lane 5 : Human lung cell lysate at 40 µg

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

Developed using the ECL technique.

Predicted band size: 150 kDa
Observed band size: 180 kDa

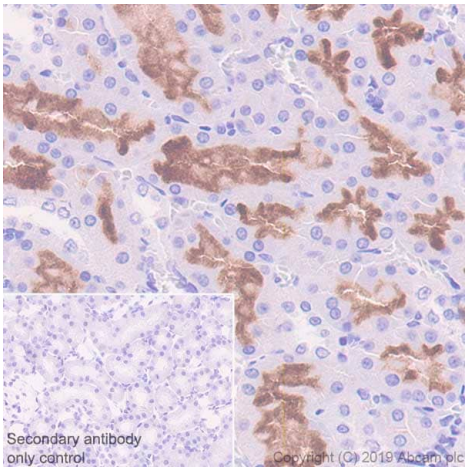
Blocking and dilution buffer: 5% NFDM/TBST.

Exposure times.

Lanes 1-3: 3 minutes. Lanes 4-5: 5.5 seconds.

ab254222 was shown to specifically react with Angiotensin Converting Enzyme 1 in wild-type HAP1 cells as signal was lost in Angiotensin Converting Enzyme 1 knockout cells. Wild-type and Angiotensin Converting Enzyme 1 knockout samples were subjected to SDS-PAGE. ab254222 and **ab181602** (Rabbit anti-GAPDH loading control) were incubated 1 hour at room temperature at 1/1000 dilution and 1/200,000 dilution respectively. Blots were developed with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) secondary antibody at 1/100,000 dilution for 1 hour at room temperature before imaging. The blot was developed on a BIO-RAD® ChemiDoc™ MP instrument using the ECL technique.

The molecular weight observed, and the expression profile are consistent with what have been described in the literature (PMID: 25495544, 16203874).



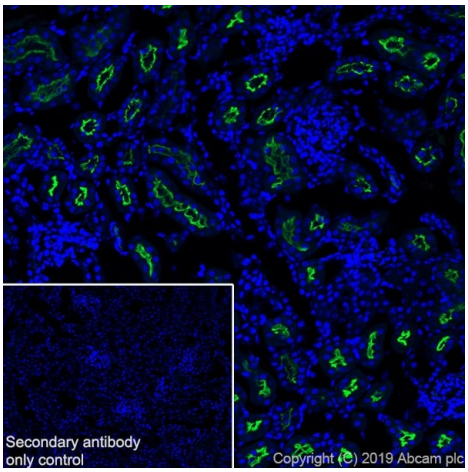
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Angiotensin Converting Enzyme 1 antibody [EPR22291-247] (ab254222)

Immunohistochemical analysis of paraffin-embedded rat kidney tissue labeling Angiotensin Converting Enzyme 1 with ab254222 at 1/4000 dilution, followed by a Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on proximal tubules of mouse kidney (PMID: 2828286; PMID: 175444) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

The section was incubated with ab254222 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

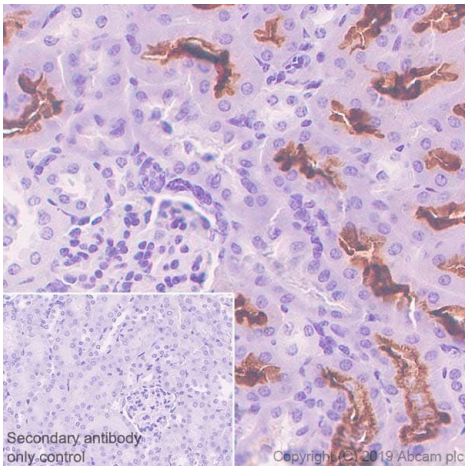


Immunohistochemistry (Frozen sections) - Anti-Angiotensin Converting Enzyme 1 antibody [EPR22291-247] (ab254222)

Immunohistochemical analysis of frozen section of 4% PFA-fixed, 0.2% Triton X-100 permeabilized mouse kidney tissue labeling Angiotensin Converting Enzyme 1 with ab254222 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution (green). Positive staining on proximal tubules of kidney (PMID:10504496) is observed. The nuclear counter stain is DAPI (blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution.

Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).



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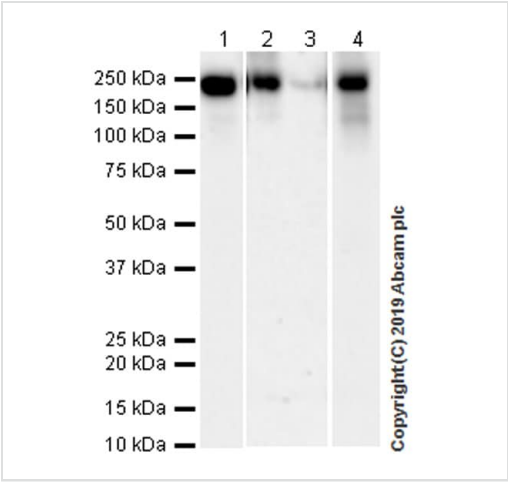
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

The section was incubated with ab254222 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Western blot - Anti-Angiotensin Converting Enzyme 1 antibody [EPR22291-247] (ab254222)

All lanes : Anti-Angiotensin Converting Enzyme 1 antibody [EPR22291-247] (ab254222) at 1/1000 dilution

Lane 1 : Rat brain tissue lysate
Lane 2 : Rat heart tissue lysate
Lane 3 : Rat liver tissue lysate
Lane 4 : Rat spleen tissue lysate

Lysates/proteins at 20 µg per lane.

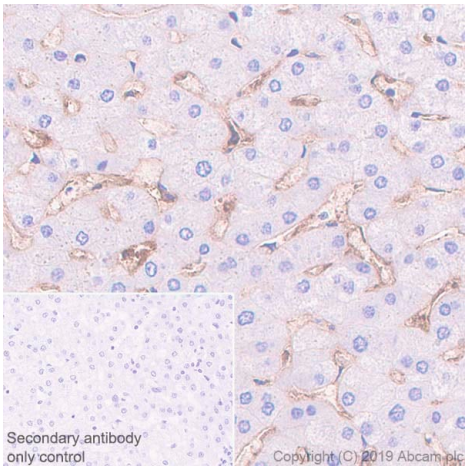
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 150 kDa
Observed band size: 180 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure times.

Lane 1: 48 seconds. Lanes 2 & 3: 3 minutes. Lane 4: 48 seconds.



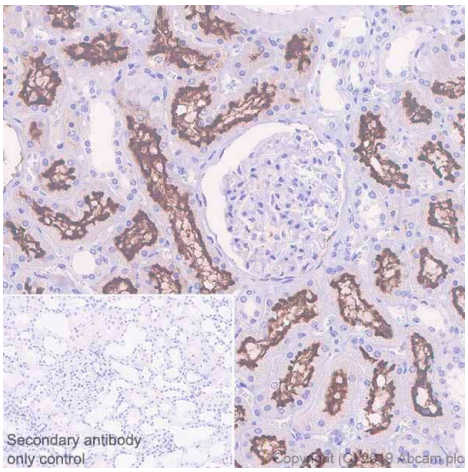
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Angiotensin Converting Enzyme 1 antibody [EPR22291-247] (ab254222)

Immunohistochemical analysis of paraffin-embedded human liver tissue labeling Angiotensin Converting Enzyme 1 with ab254222 at 1/4000 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on blood vessels of human liver (PMID: 175444) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

The section was incubated with ab254222 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



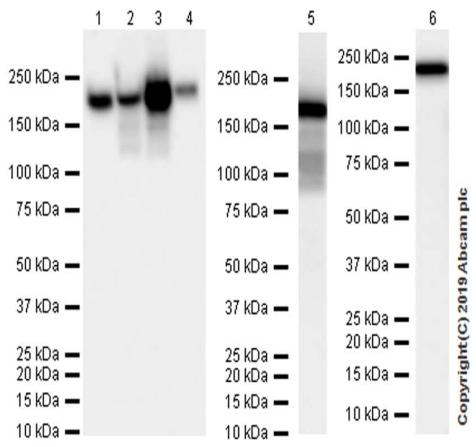
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Angiotensin Converting Enzyme 1 antibody [EPR22291-247] (ab254222)

Immunohistochemical analysis of paraffin-embedded human lkidney tissue labeling Angiotensin Converting Enzyme 1 with ab254222 at 1/4000 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Positive staining on proximal tubules of human kidney (PMID: 2828286; PMID: 175444) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.

The section was incubated with ab254222 for 30 mins at room temperature.
The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Western blot - Anti-Angiotensin Converting Enzyme 1 antibody [EPR22291-247] (ab254222)

All lanes : Anti-Angiotensin Converting Enzyme 1 antibody [EPR22291-247] (ab254222) at 1/1000 dilution

Lane 1 : Mouse brain tissue lysate
Lane 2 : Mouse heart tissue lysate
Lane 3 : Mouse kidney tissue lysate
Lane 4 : Mouse spleen tissue lysate
Lane 5 : Mouse lung tissue lysate
Lane 6 : bEND.3 (mouse brain endothelioma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

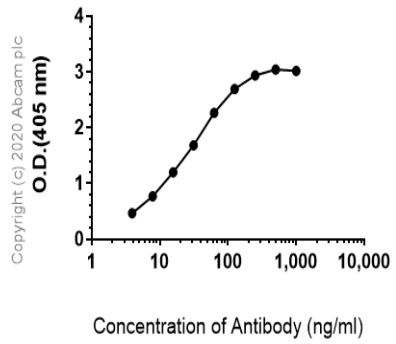
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 150 kDa
Observed band size: 180 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

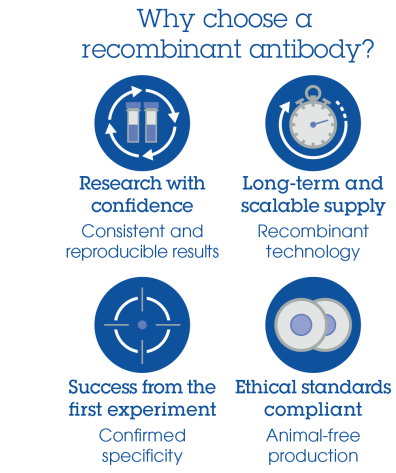
Exposure times.

Lanes 1-4: 10 seconds. Lane 5: 5.5 seconds. Lane 6: 48 seconds.



Indirect ELISA - Anti-Angiotensin Converting Enzyme 1 antibody [EPR22291-247] (ab254222)

ELISA analysis of ACE recombinant protein at 1000 ng/mL with ab254222. An Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) at 1/2500 dilution was used as the secondary antibody.



Anti-Angiotensin Converting Enzyme 1 antibody [EPR22291-247] (ab254222)

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