abcam

Product datasheet

Anti-RAGE antibody [EPR21171] ab216329

Recombinant RabMAb

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Overview

Product name	Anti-RAGE antibody [EPR21171]
Description	Rabbit monoclonal [EPR21171] to RAGE
Host species	Rabbit
Specificity	RAGE is typically expressed at low levels under normal physiological conditions in majority of tissues except normal lung tissue. When testing other tissues, please use lung tissue as a positive control.
Tested applications	**Suitable for:** IP, WB, IHC-P, IHC-Fr, ICC/IF, Flow Cyt
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Human fetal lung lysate; Mouse and rat lung lysates. IHC-P: Human, mouse and rat lung tissues. IHC-Fr: Mouse and rat lung tissues. ICC/IF: HEK-293T cells. Flow Cyt: HEK-293T cells. IP: Mouse lung 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. 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	EPR21171
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab216329 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
IP		1/30.
WB	★★★★☆ (1)	1/1000. Predicted molecular weight: 42 kDa.
IHC-P		1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IHC-Fr		1/500. Perform heat mediated antigen retrieval by using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20) before commencing with IHC staining protocol.
ICC/IF		1/100.
Flow Cyt		1/500.

Target

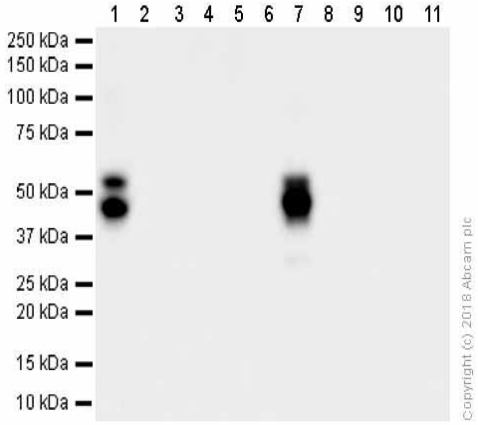
Function Mediates interactions of advanced glycosylation end products (AGE). These are nonenzymatically glycosylated proteins which accumulate in vascular tissue in aging and at an accelerated rate in diabetes. Acts as a mediator of both acute and chronic vascular inflammation in conditions such as atherosclerosis and in particular as a complication of diabetes. AGE/RAGE signaling plays an important role in regulating the production/expression of TNF-alpha, oxidative stress, and endothelial dysfunction in type 2 diabetes. Interaction with S100A12 on endothelium, mononuclear phagocytes, and lymphocytes triggers cellular activation, with generation of key proinflammatory mediators. Interaction with S100B after myocardial infarction may play a role in myocyte apoptosis by activating ERK1/2 and p53/TP53 signaling (By similarity). Receptor for amyloid beta peptide. Contributes to the translocation of amyloid-beta peptide (ABPP) across the cell membrane from the extracellular to the intracellular space in cortical neurons. ABPP-initiated RAGE signaling, especially stimulation of p38 mitogen-activated protein kinase (MAPK), has the capacity to drive a transport system delivering ABPP as a complex with RAGE to the intraneuronal space.

Tissue specificity Endothelial cells.

Sequence similarities Contains 2 Ig-like C2-type (immunoglobulin-like) domains.
Contains 1 Ig-like V-type (immunoglobulin-like) domain.

Cellular localization Secreted and Cell membrane.

Images



Western blot - Anti-RAGE antibody [EPR21171] (ab216329)

All lanes : Anti-RAGE antibody [EPR21171] (ab216329) at 1/2000 dilution

Lane 1 : Mouse lung lysates with 5% NFDM/TBST
Lane 2 : Mouse brain lysates with 5% NFDM/TBST
Lane 3 : Mouse kidney lysates with 5% NFDM/TBST
Lane 4 : Mouse heart lysates with 5% NFDM/TBST
Lane 5 : Mouse liver lysates with 5% NFDM/TBST
Lane 6 : Mouse spleen lysates with 5% NFDM/TBST
Lane 7 : Rat lung lysates with 5% NFDM/TBST
Lane 8 : Rat brain lysates with 5% NFDM/TBST
Lane 9 : Rat kidney lysates with 5% NFDM/TBST
Lane 10 : Rat heart lysates with 5% NFDM/TBST
Lane 11 : Rat spleen lysates with 5% NFDM/TBST

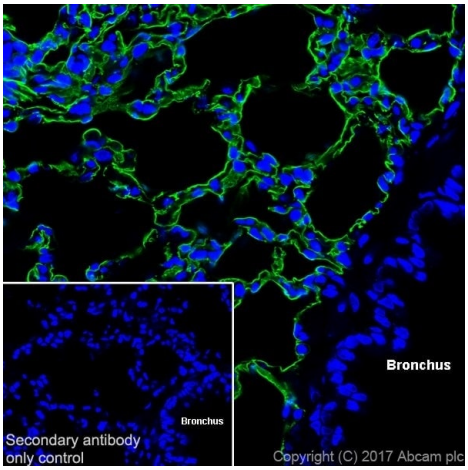
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 42 kDa
Observed band size: 43 kDa

Exposure time: 3 seconds

The expression profile and molecular mass observed is consistent with what has been described in the literature (PMID:16315007; PMID:18355449; PMID:18245812)

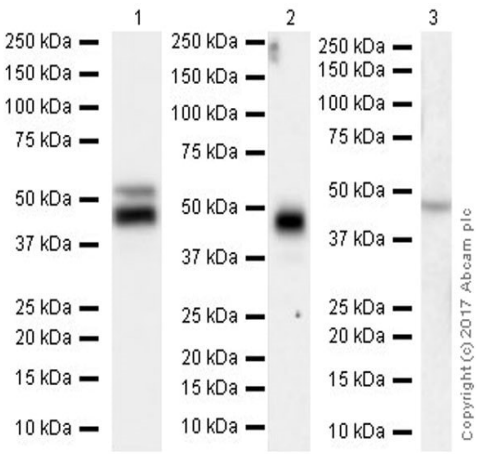


Immunohistochemistry (Frozen sections) - Anti-RAGE antibody [EPR21171] (ab216329)

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen rat lung tissue labeling RAGE with ab216329 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Positive membrane staining on alveolar epithelial cells, negative on the bronchial epithelial cells on rat lung tissue section is observed (PMID: 15173891).

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.



Western blot - Anti-RAGE antibody [EPR21171] (ab216329)

All lanes : Anti-RAGE antibody [EPR21171] (ab216329) at 1/1000 dilution

Lane 1 : Mouse lung lysate
Lane 2 : Rat lung lysate
Lane 3 : Human fetal lung lysate

Lysates/proteins at 10 µg per lane.

Secondary
Lanes 1-2 : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution
Lane 3 : VeriBlot for IP Detection Reagent (HRP) (**ab131366**) at 1/4000 dilution

Developed using the ECL technique.

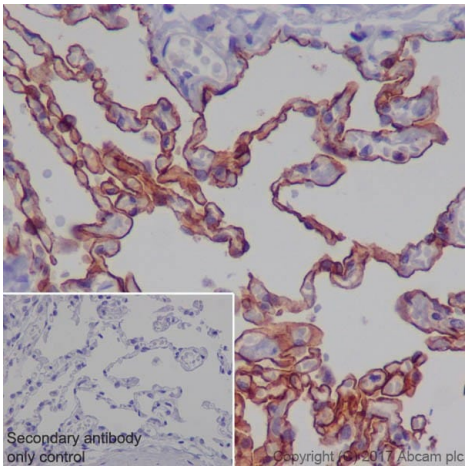
Predicted band size: 42 kDa
Observed band size: 45,55 kDa

Exposure times: Lane 1: 5 seconds; Lane 2: 10 seconds; Lane 3: 3 minutes.

Blocking/Dilution buffer: 5% NFDM/TBST.

The blot was developed on a BIO-RAD® ChemiDoc™ MP instrument.

The expression profile and molecular mass observed is consistent with what has been described in the literature (PMID:16315007; PMID:18355449; PMID:18245812). Full-length RAGE is not detected in rat and human lysates.

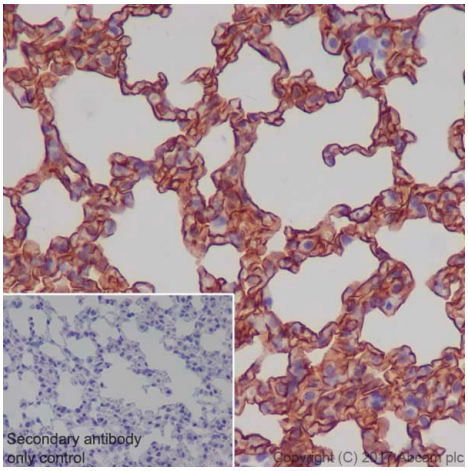


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RAGE antibody [EPR21171] (ab216329)

Immunohistochemical analysis of paraffin-embedded human lung tissue labeling RAGE with ab216329 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Membranous staining on epithelial cells of human lung (PMID: 19592063; PMID: 26472810) is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

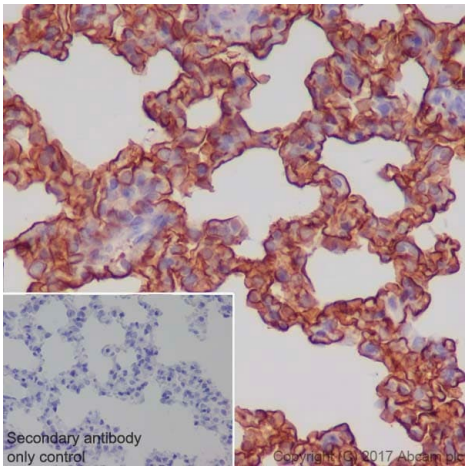


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RAGE antibody [EPR21171] (ab216329)

Immunohistochemical analysis of paraffin-embedded mouse lung tissue labeling RAGE with ab216329 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Mainly membranous staining on epithelial cells of mouse lung (PMID: 19592063; PMID: 26472810) is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

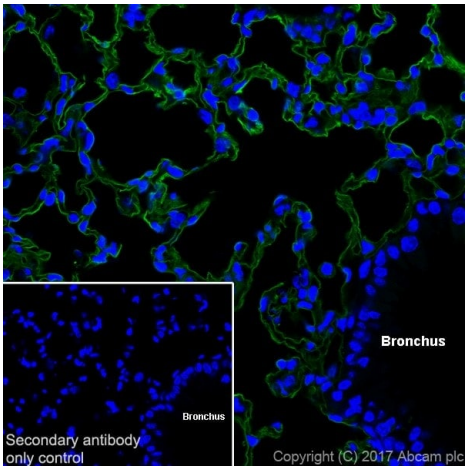


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RAGE antibody [EPR21171] (ab216329)

Immunohistochemical analysis of paraffin-embedded rat lung tissue labeling RAGE with ab216329 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Mainly membranous staining on epithelial cells of rat lung (PMID: 19592063; PMID: 26472810) is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

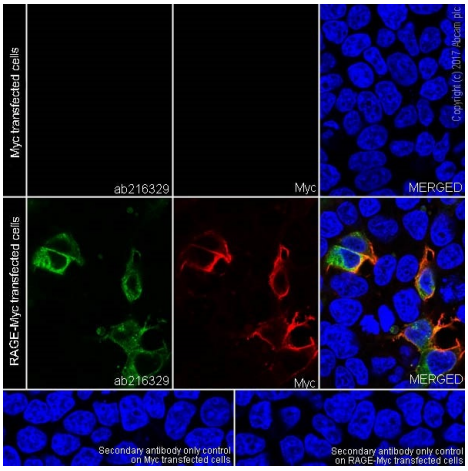


Immunohistochemistry (Frozen sections) - Anti-RAGE antibody [EPR21171] (ab216329)

Immunohistochemical analysis of 4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized frozen mouse lung tissue labeling RAGE with ab216329 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Positive membrane staining on alveolar epithelial cells, negative on the bronchial epithelial cells on mouse lung tissue section is observed (PMID: 15173891).

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.



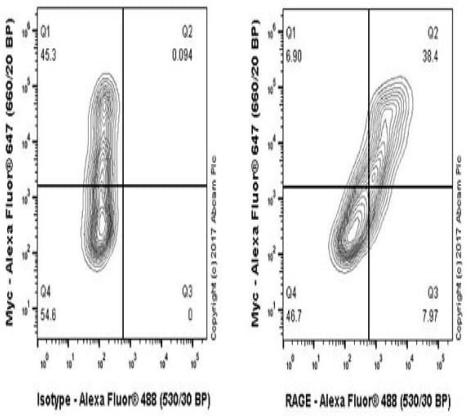
Immunocytochemistry/ Immunofluorescence - Anti-RAGE antibody [EPR21171] (ab216329)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100-permeabilized HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cells transfected with Myc-tagged RAGE expression vector labeling RAGE with ab216329 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing positive staining in HEK-293T cells transfected with Myc-tagged RAGE expression vector.

The nuclear counter stain is DAPI (blue). Myc-Tag is detected with Myc-Tag (9B11) Mouse mAb (Alexa Fluor® 647 Conjugate) (red) at 1/1000 dilution.

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

Negative control: Myc-transfected HEK-293T cells.



Flow Cytometry - Anti-RAGE antibody [EPR21171] (ab216329)

Flow cytometric analysis of 4% paraformaldehyde-fixed HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) cells transfected with Myc-tagged RAGE expression vector labeling RAGE with ab216329 at 1/500 dilution (right panel) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (left panel). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/2000 dilution was used as the secondary antibody.

Fresh cells were surface-stained with **ab172730** and ab216329 respectively. Then fixed with 2% PFA for 15min and intracellular stained with anti-Myc tag antibody (Y axis). Only Myc+ population give positive signal.

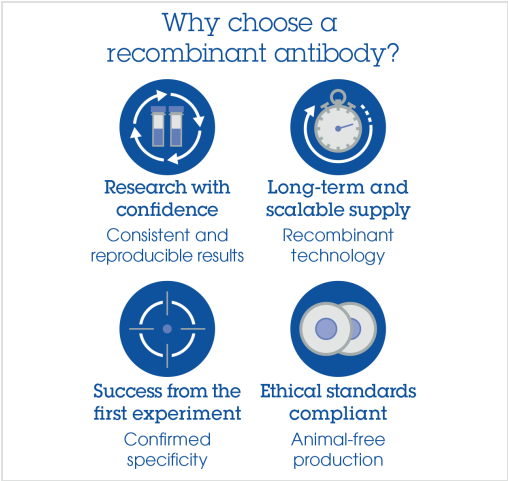
Immunoprecipitation - Anti-RAGE antibody [EPR21171] (ab216329)

RAGE was immunoprecipitated from 0.35 mg of mouse lung lysate with ab216329 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab216329 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10,000 dilution

Lane 1: Mouse lung lysate 10 µg (Input).
Lane 2: ab216329 IP in mouse lung lysate.
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab216329 in mouse lung lysate.

Exposure time: 10 seconds.

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



Anti-RAGE antibody [EPR21171] (ab216329)

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

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