abcam

ab228861

Anti-RAGE antibody [EPR21171] - BSA and Azide free

Recombinant | RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR21171
Purification technique	Affinity purification Protein A
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. FURTHER INFORMATION ON SPECIFICITY (Chinese Version) available under the product protocols section. This file includes key technical notes of experience when using this product.
Concentration	1.041 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Mouse
Dilution info	-
Notes	-

Expected

Species	Human, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	-
Notes	Endogenous RAGE expression in cell lines is low; therefore, antibody performance in natural cell line samples has not been validated. This antibody has been validated only in RAGE-overexpressing cell lines.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse, Rat, Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	Endogenous RAGE expression in cell lines is low; therefore, antibody performance in natural cell line samples has not been validated. This antibody has been validated only in RAGE-overexpressing cell lines.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-Fr

Tested

Species	Rat
Dilution info	-
Notes	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.

Species	Mouse
Dilution info	-
Notes	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.

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

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

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

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

Target data

See full target information AGER

Function

Cell surface pattern recognition receptor that senses endogenous stress signals with a broad ligand repertoire including advanced glycation end products, S100 proteins, high-mobility group box 1 protein/HMGB1, amyloid beta/APP oligomers, nucleic acids, histones, phospholipids and glycosaminoglycans (PubMed:27572515, PubMed:28515150, PubMed:34743181, PubMed:35974093, PubMed:24081950). Advanced glycosylation end products are nonenzymatically glycosylated proteins which accumulate in vascular tissue in aging and at an accelerated rate in diabetes (PubMed:21565706). These ligands accumulate at inflammatory sites during the pathogenesis of various diseases including diabetes, vascular complications, neurodegenerative disorders and cancers, and RAGE transduces their binding into pro-inflammatory responses. Upon ligand binding, uses TIRAP and MYD88 as adapters to transduce the signal ultimately leading to the induction of inflammatory cytokines IL6, IL8 and TNFalpha through activation of NF-kappa-B (PubMed:21829704, PubMed:33436632). Interaction with S100A12 on endothelium, mononuclear phagocytes, and lymphocytes triggers cellular activation, with generation of key pro-inflammatory mediators (PubMed:19386136). Interaction with S100B after myocardial infarction may play a role in myocyte apoptosis by activating ERK1/2 and p53/TP53 signaling (By similarity). Contributes to the translocation of amyloid-beta peptide (ABPP) across the cell membrane from the extracellular to the intracellular space in cortical neurons (PubMed:19906677). 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. Participates in endothelial albumin transcytosis together with HMGB1 through the RAGE/SRC/Caveolin-1 pathway, leading to endothelial hyperpermeability (PubMed:27572515). Mediates the loading of HMGB1 in extracellular vesicles (EVs) that shuttle HMGB1 to hepatocytes by transferrin-mediated endocytosis and

subsequently promote hepatocyte pyroptosis by activating the NLRP3 inflammasome (PubMed:34743181). Binds to DNA and promotes extracellular hypomethylated DNA (CpG DNA) uptake by cells via the endosomal route to activate inflammatory responses (PubMed:24081950, PubMed:28515150). Mediates phagocytosis by non-professional phagocytes (NPP) and this is enhanced by binding to ligands including RNA, DNA, HMGB1 and histones (PubMed:35974093). Promotes NPP-mediated phagocytosis of Saccharomyces cerevisiae spores by binding to RNA attached to the spore wall (PubMed:35974093). Also promotes NPP-mediated phagocytosis of apoptotic cells (PubMed:35974093). Following DNA damage, recruited to DNA double-strand break sites where it colocalizes with the MRN repair complex via interaction with double-strand break repair protein MRE11 (By similarity). Enhances the endonuclease activity of MRE11, promoting the end resection of damaged DNA (By similarity). Promotes DNA damage repair in trophoblasts which enhances trophoblast invasion and contributes to placental development and maintenance (PubMed:33918759). Protects cells from DNA replication stress by localizing to damaged replication forks where it stabilizes the MCM2-7 complex and promotes faithful progression of the replication fork (PubMed:36807739). Mediates the production of reactive oxygen species (ROS) in human endothelial cells (PubMed:25401185).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab228861 is the carrier-free version of ab216329.

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.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	RAGE also known as Receptor for Advanced Glycation End-products is a multi-ligand cell surface receptor with a molecular weight of approximately 45 kDa. It belongs to the immunoglobulin superfamily consisting of three extracellular immunoglobulin-like domains a transmembrane domain and a cytoplasmic tail. RAGE is widely expressed in various tissues throughout the body with high expression levels in the lungs heart and cells of the nervous system. The receptor can interact with several ligands such as advanced glycation end-products (AGEs) amyloid beta and S100/calgranulin proteins facilitating signal transduction into the cells.
Biological function summary	RAGE functions in the immune and inflammatory response where it mediates cell signaling that leads to cellular activation and the release of pro-inflammatory cytokines. It acts as part of complexes with different proteins contributing to cellular processes such as proliferation and migration. RAGE also plays roles in the regulation of oxidative stress and apoptosis impacting cellular health and survival. Researchers employ tools like 'anti-RAGE' antibodies and 'RAGER ELISA' assays to measure and study RAGE expression levels and its interactions in various experimental setups.
Pathways	RAGE is significantly involved in the NF-kB pathway and the MAPK signaling cascade. Its activation can lead to the release of NF-kB a transcription factor that plays an essential role in immune and inflammatory responses. RAGE interacts with proteins such as p38 MAPK leading to a cascade of events that regulate inflammation and stress responses. The signaling pathways involving RAGE are important in maintaining cell homeostasis and responding to cellular stressors and tools like 'anti-RAGE' and 'mouse RAGE' antibodies serve to elucidate these complex pathways further.
Associated diseases and disorders	RAGE has strong associations with chronic diseases like diabetes and Alzheimer's disease. In diabetes RAGE binds to AGEs contributing to inflammation and vascular complications where it often interacts with proteins like iNOS and VEGF. In Alzheimer's disease RAGE is implicated in the accumulation and toxicity of amyloid-beta peptides interacting with proteins such as APP and tau. Understanding RAGE's role in these diseases can aid in developing therapeutic

strategies employing reagents such as 'phen RAGE' and 'anti-RAGE' for targeted treatment approaches.

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

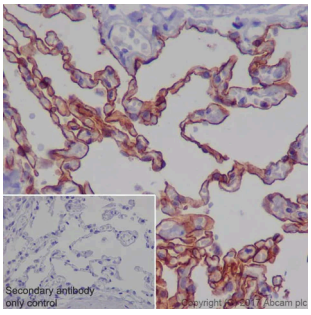
We do not recommend this combination. It is not covered by our product promise.

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.

11 product images



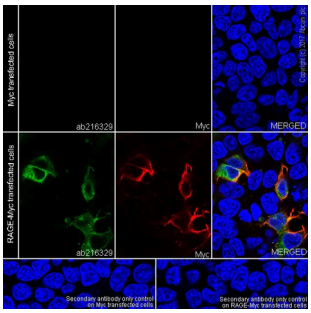
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RAGE antibody [EPR21171] - BSA and Azide free (ab228861)

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

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab216329).

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



Immunocytochemistry/ Immunofluorescence - Anti-RAGE antibody [EPR21171] - BSA and Azide free (ab228861)

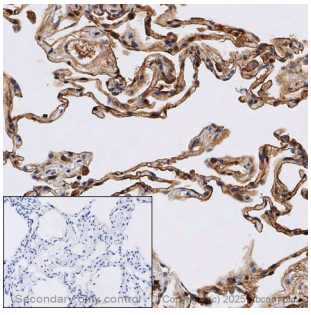
RAGE Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-RAGE antibody

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab216329).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RAGE antibody [EPR21171] - BSA and Azide free (ab228861)

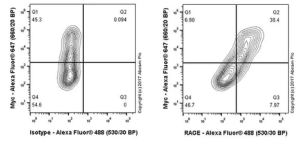
RAGE Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human lung tissue using rabbit Anti-RAGE antibody

This data was developed using ab216329, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of formalin fixed paraffin embedded human lung labelling RAGE with ab216329 at a concentration of 0.05µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with a OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was performed with DISCOVERY cell conditioning solution (CC1) 100°C, pH8.5 for 32mins.

ab216329 anti-RAGE antibody [EPR21171] was incubated for 16mins at 37°C. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

Customers are encouraged to optimise antigen retrieval conditions, antibody concentration, incubation times and temperature for best results in their own IHC assay workflow (automated and manual)

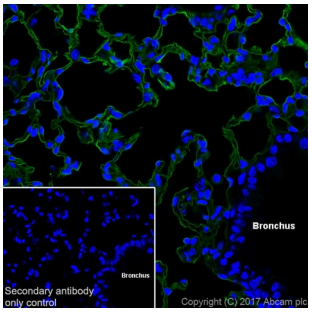


Flow Cytometry - Anti-RAGE antibody [EPR21171] - BSA and Azide free (ab228861)

RAGE Flow Cytometry staining using rabbit Anti-RAGE antibody

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab216329).



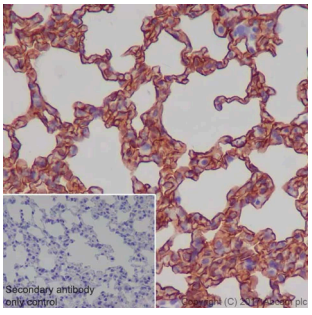
Immunohistochemistry (Frozen sections) - Anti-RAGE antibody [EPR21171] - BSA and Azide free (ab228861)

RAGE Immunohistochemistry (Frozen sections) staining using rabbit Anti-RAGE antibody

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab216329).



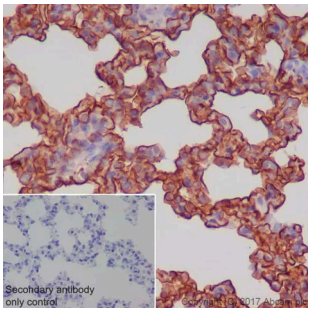
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RAGE antibody [EPR21171] - BSA and Azide free (ab228861)

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

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab216329).

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



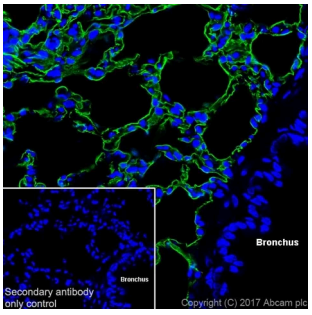
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RAGE antibody [EPR21171] - BSA and Azide free (ab228861)

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

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab216329).

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



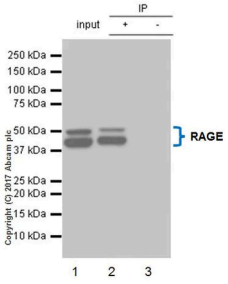
Immunohistochemistry (Frozen sections) - Anti-RAGE antibody [EPR21171] - BSA and Azide free (ab228861)

RAGE Immunohistochemistry (Frozen sections) staining using rabbit Anti-RAGE antibody

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab216329).



Immunoprecipitation - Anti-RAGE antibody [EPR21171] - BSA and Azide free (ab228861)

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 1/10000 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.

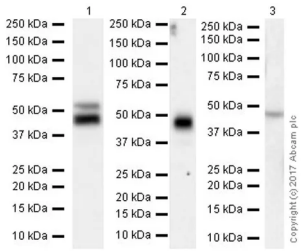
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab216329).

All lanes:
Immunoprecipitation - Anti-RAGE antibody [EPR21171] (ab216329)

Developed using the ECL technique.

Predicted band size: 42 kDa

Observed band size: 45 kDa, 55 kDa



Western blot - Anti-RAGE antibody [EPR21171] - BSA and Azide free (ab228861)

RAGE Western blot staining using rabbit Anti-RAGE antibody

This data was developed using ab216329, the same antibody clone in a different buffer formulation.
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.

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

Lane 1:
Mouse lung lysate at 10 µg

Lane 2:
Rat lung lysate at 10 µg

Lane 3:
Human fetal lung lysate at 10 µg

Secondary

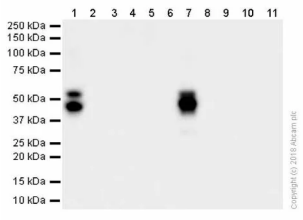
Lanes 1 - 2:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Lane 3:
Western blot - 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 kDa



Western blot - Anti-RAGE antibody [EPR21171] - BSA and Azide free (ab228861)

RAGE Western blot staining using rabbit Anti-RAGE antibody

This data was developed using ab216329, the same antibody clone in a different buffer formulation.
The expression profile and molecular mass observed is consistent with what has been described in the literature (PMID:16315007; PMID:18355449; PMID:18245812).

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

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

Lane 1:
Mouse lung lysates at 20 µg

Lane 2:
Mouse brain lysates at 20 µg

Lane 3:
Mouse kidney lysates at 20 µg

Lane 4:
Mouse heart lysates at 20 µg

Lane 5:
Mouse liver lysates at 20 µg

Lane 6:
Mouse spleen lysates at 20 µg

Lane 7:
Rat lung lysates at 20 µg

Lane 8:
Rat brain lysates at 20 µg

Lane 9:
Rat kidney lysates at 20 µg

Lane 10:
Rat heart lysates at 20 µg

Lane 11:
Rat spleen lysates at 20 µg

Secondary

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

Predicted band size: 42 kDa

Observed band size: 43 kDa

Exposure time: 3s

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.