Anti-RAGE Antibody [JF0975] - BSA and Azide free

HA750341

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Tissue
Molecular Wt:	Predicted band size: 43 kDa
Clone number:	JF0975

Description: Advanced glycosylation end products of proteins (AGEs) are nonenzymatically glycosylated proteins that are associated with a variety of conditions, including diabetes and other vascular disorders, as well as amyloidosis. These proteins regulate cellular functions via specific cell surface acceptor molecules, such as RAGE (receptor for advanced glycosylation end products). RAGE is a type 1 membrane protein that is found on the surface of endothelial cells, mononuclear phagocytes and vascular smooth muscle cells. Binding of AGEs to RAGE results in the induction of cellular oxidant stress and activation of the transcription factor NFkB. Evidence suggests that the induction of oxidant stress results in the activation of an intracellular cascade involving p21 ras and MAP kinase, which leads to activation of transcription.

Immunogen: Synthetic peptide within human RAGE aa 350-390.

Positive control: Mouse lung tissue lysate, rat lung tissue lysate, rat lung tissue, mouse lung tissue, human lung tissue.

Subcellular location: Cell membrane, Secreted.

Database links: SwissProt: Q15109 Human | Q62151 Mouse | Q63495 Rat

Recommended Dilutions:

WB	1:2,000-1:5,000
IHC-P	1:200-1:1,000
IF-Tissue	1:100
mIHC	1:3,000

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

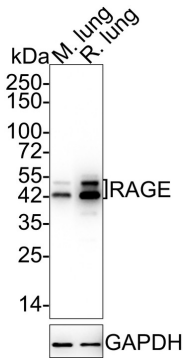

Fig1: Western blot analysis of RAGE on different lysates with Rabbit anti-RAGE antibody (HA750341) at 1/5,000 dilution.

Lane 1: Mouse lung tissue lysate
Lane 2: Rat lung tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 43 kDa
Observed band size: 43/50 kDa

Exposure time: 24 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750341) at 1/5,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

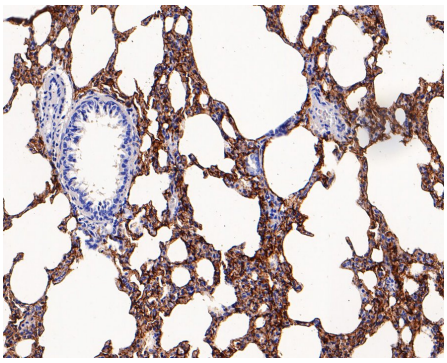

Fig2: Immunohistochemical analysis of paraffin-embedded rat lung tissue with Rabbit anti-RAGE antibody (HA750341) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750341) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

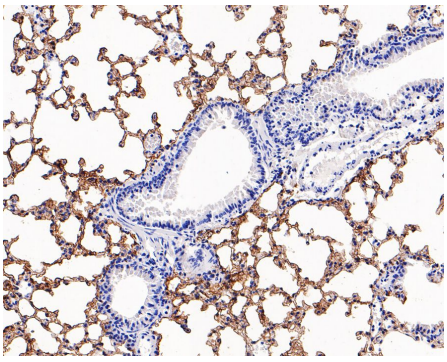

Fig3: Immunohistochemical analysis of paraffin-embedded mouse lung tissue with Rabbit anti-RAGE antibody (HA750341) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750341) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

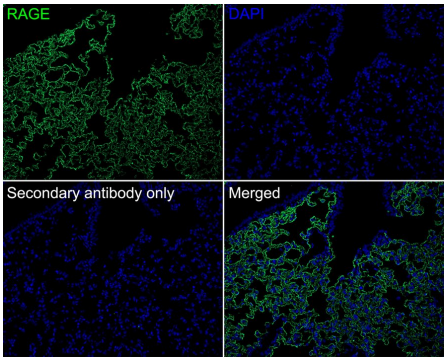

Fig4: Immunofluorescence analysis of paraffin-embedded rat lung tissue (perfusion) labeling RAGE with Rabbit anti-RAGE antibody (HA750341) at 1/100 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA750341, green) at 1/100 dilution overnight at 4 ℃, washed with PBS.

Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

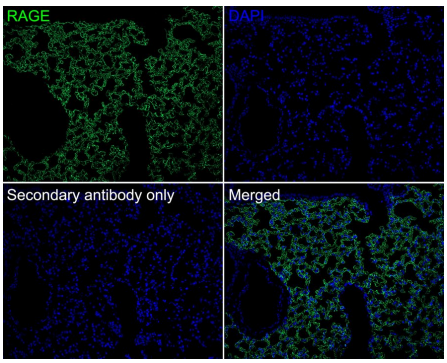

Fig5: Immunofluorescence analysis of paraffin-embedded mouse lung tissue (perfusion) labeling RAGE with Rabbit anti-RAGE antibody (HA750341) at 1/100 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA750341, green) at 1/100 dilution overnight at 4 ℃, washed with PBS.

Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

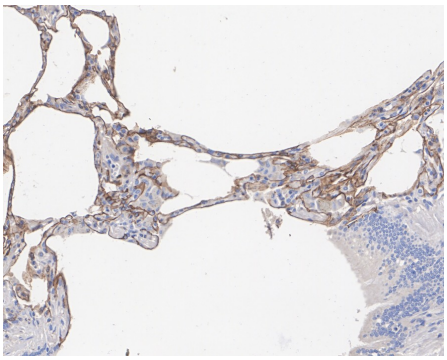

Fig6: Immunohistochemical analysis of paraffin-embedded human lung tissue with Rabbit anti-RAGE antibody (HA750341) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750341) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

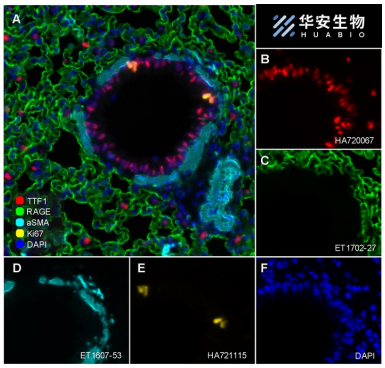

Fig7: Fluorescence multiplex immunohistochemical analysis of mouse lung (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-TTF1 (HA720067, Red), anti-RAGE (HA750341, Green), anti-aSMA (ET1607-53, Cyan) and anti-Ki67 (HA721115, Yellow) on mouse lung. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in four rounds of staining: in the order of HA720067 (1/4,000 dilution), ET1702-27 (1/3,000 dilution), ET1607-53 (1/10,000 dilution) and HA721115 (1/3,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Olympus VS200 Slide Scanner.

Background References

1. Kim J et al. Cytoplasmic translocation of high-mobility group box-1 protein is induced by diabetes and high glucose in retinal pericytes. Mol Med Rep 14:3655-61 (2016).
2. Yao X et al. Mitochondrial ROS Induces Cardiac Inflammation via a Pathway through mtDNA Damage in a Pneumonia-Related Sepsis Model. PLoS One 10:e0139416 (2015).