Human/Mouse/Rat RAGE Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF1145

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human RAGE in direct ELISAs and Western blots. In direct ELISAs, approximately 25% cross-reactivity recombinant canine RAGE is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human RAGE Gln24-Ala344 Accession # Q15109
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.

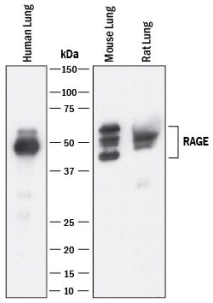
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Immunohistochemistry	0.5-15 µg/mL	See Below
Blockade of Receptor-ligand Interaction	In a functional ELISA, 4-12 µg/mL of this antibody will block 50% of the binding of 500 ng/mL of biotinylated AGE-BSA to immobilized Recombinant Human RAGE Fc Chimera (Catalog # 1145-RG) coated at 5 µg/mL (100 µL/well). At 80 µg/mL, this antibody will block >90% of the binding.	

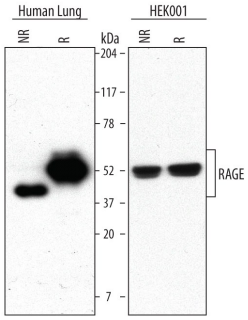
DATA

Western Blot



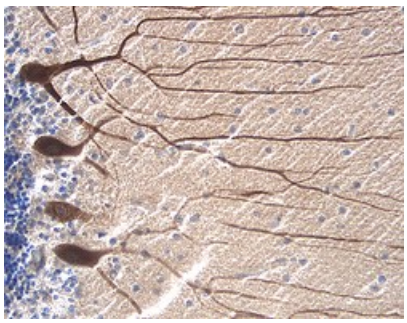
Detection of Human, Mouse, and Rat RAGE by Western Blot. Western blot shows lysates of human, mouse, and rat lung tissue. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human/Mouse/Rat RAGE Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1145) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). Specific bands were detected for RAGE at approximately 45-55 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Western Blot



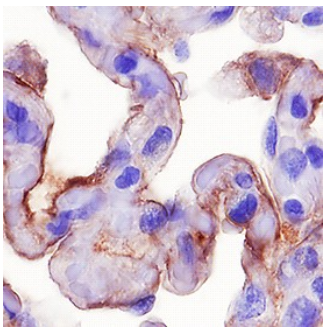
Detection of Human RAGE by Western Blot. Western blot shows lysates of human lung tissue and HEK001 human epidermal keratinocyte cell line. PVDF Membrane was probed with 1 µg/mL of Goat Anti-Human/Mouse/Rat RAGE Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1145) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). Specific bands were detected for RAGE at approximately 43 kDa under non-reducing (NR) conditions and 50 kDa under reducing (R) conditions (as indicated). This experiment was conducted using Immunoblot Buffer Group 5.

Immunohistochemistry



RAGE in Human Alzheimer's Disease Brain. RAGE was detected in immersion fixed paraffin-embedded sections of human Alzheimer's disease brain (cerebellum) using 15 µg/mL Goat Anti-Human/Mouse/Rat RAGE Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1145) overnight at 4 °C. Tissue was stained with the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Immunohistochemistry



RAGE in Human Lung. RAGE was detected in immersion fixed paraffin-embedded sections of human lung using Goat Anti-Human/Mouse/Rat RAGE Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1145) at 0.5 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC004). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cell membranes. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

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PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Advanced glycation endproducts (AGE) are adducts formed by the non-enzymatic glycation or oxidation of macromolecules. AGE forms during aging and its formation is accelerated under pathophysiologic states such as diabetes, Alzheimer's disease, renal failure and immune/inflammatory disorders. Receptor for Advanced Glycation Endoproducts (RAGE), named for its ability to bind AGE, is a multi-ligand receptor belonging the immunoglobulin (Ig) superfamily. Besides AGE, RAGE binds amyloid β-peptide, S100/calgranulin family proteins, high mobility group B1 (HMGB1, also know as amphoterin) and leukocyte integrins.

The human RAGE gene encodes a 404 amino acid residues (aa) type I transmembrane glycoprotein with a 22 aa signal peptide, a 320 aa extracellular domain containing an Ig-like V-type domain and two Ig-like Ce-type domains, a 21 aa transmembrane domain and a 41 aa cytoplasmic domain. The V-type domain and the cytoplasmic domain are important for ligand binding and for intracellular signaling, respectively. Two alternative splice variants, lacking the V-type domain or the cytoplasmic tail, are known. RAGE is highly expressed in the embryonic central nervous system. In adult tissues, RAGE is expressed at low levels in multiple tissues including endothelial and smooth muscle cells, mononuclear phagocytes, pericytes, microglia, neurons, cardiac myocytes, and hepatocytes. The expression of RAGE is upregulated upon ligand interaction. Depending on the cellular context and interacting ligand, RAGE activation can trigger differential signaling pathways that affect divergent pathways of gene expression. RAGE activation modulates varied essential cellular responses (including inflammation, immunity, proliferation, cellular adhesion, and migration) that contribute to cellular dysfunction associated with chronic diseases such as diabetes, cancer, amyloidoses, and immune or inflammatory disorders.

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