Human RAGE Mouse Monoclonal Antibody (Clone KH-2G12)

Cat# CY-M1041

100 µg (1.0 mg/mL x 100 µL)

Clone Name	Applications	Species Cross-Reactivity	Molecular Wt.	Source Isotype
KH-2G12	WB, F	H	43-54 kDa	Mouse IgG1

Background

RAGE is a multi-ligand member of the immunoglobulin superfamily of cell surface molecules that is expressed in a variety of cell lines, including endothelial cells, smooth muscle cells, mononuclear phagocytes, pericytes, neurons, cardiac myocytes, mesangial cells and hepatocytes (1, 2). RAGE interacts with different structures to transmit a signal into the cell and recognizes three-dimensional structures rather than specific amino acid sequences. Therefore, RAGE seems to fulfill the requirements of a pattern-recognition receptor. As a member of the immunoglobulin superfamily, it interacts with a diverse class of ligands, including AGEs (3, 4), HMGB1 (also known as Amphoterin) (5), amyloid β-peptide (6), amyloid A (7), leukocyte adhesion receptors (8), prions (9), Escherichia coli curli operons (10), β-sheet fibrils (11) and several members of the S100 protein superfamily including S100/calgranulins (12). Thus RAGE may have potential involvement in several pathological processes including inflammation, diabetes, Alzheimer's disease (AD), systemic amyloidosis, and tumor growth (13).

Specificity/Sensitivity: The Human RAGE Monoclonal Antibody detects overexpressed RAGE by flow cytometory and western blotting.

Source/Purification: The antibody is produced by immunizing mice with a recombinant extacellular domain of human RAGE. IgG is purified by protein A-Sepharose chromatography.

Recommended Antibody Dilutions: Western blotting: 0.5-1 µg/mL

Storage: Supplied in 20 mM phosphate buffer (pH 7.5), 300 mM NaCl, 50 % glycerol. Store at –20°C.

Applications Key: WB: Western **IP:** Immunoprecipitation **IHC:** Immunohistochemistry **IC:** Immunocytochemistry **F:** Flow cytometry **E:** ELISA **FP:** Fluorescence Polarization assay
Species Cross-Reactivity Key: H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Mi:** mink **C:** chicken **X:** *Xenopus* **Z:** zebra fish **All:** all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

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General References:

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4. Schmidt AM, Vianna M, Gerlach M, et al. J Biol Chem 1992, 267: 14987-14997

5. Hori O, Brett J, Slattery T, Cao R et al. J Biol Chem 1995, 270: 25752-25761.

6. Yan SD, Zhu H, Fu J, Yan SF, Roher A et al. Proc Natl Acad Sci U S A 1997, 94: 5296-5301.

7. Yan SD, Zhu H, Zhu A, Golabek A, Du H, Roher A, Yu J, Soto C, Schmidt AM, Stern D, et al. Nat Med 2000, 6: 643-651.

8. Chavakis T, Bierhaus A, Al-Fakhri N et al. J Exp Med 2003, 198: 1507-1515.

9. Sasaki N, Takeuchi M, Chowei H, Kikuchi S et al Neurosci Lett. 2002, 326: 117-120.

10. Chapman MR, Robinson LS, Pinkner JS et al. Science 2002, 295: 851-855

11. Bierhaus A, Humpert PM, Morcos M, et al. J Mol Med 2005, 83: 876- 886.

12. Hofmann MA, Drury S, Fu C, Qu W et al. Cell 1999, 97: 889-901.

13. Stern DM, Yan SD, Yan SF, and Schmidt AM. Ageing Res Rev. 2002, 1: 1-15.

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Fig.1 Flow cytometry analysis of RAGE/HEK293 Cell Line (CY-C8250) by using anti-RAGE monoclonal antibody, KH-2G12 (CY-M1041)

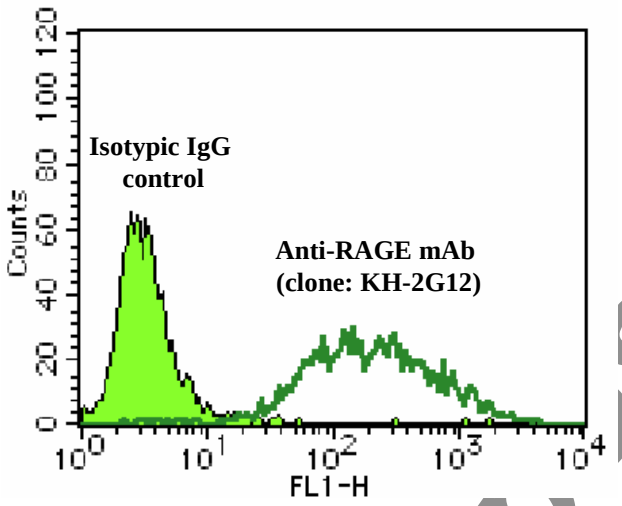
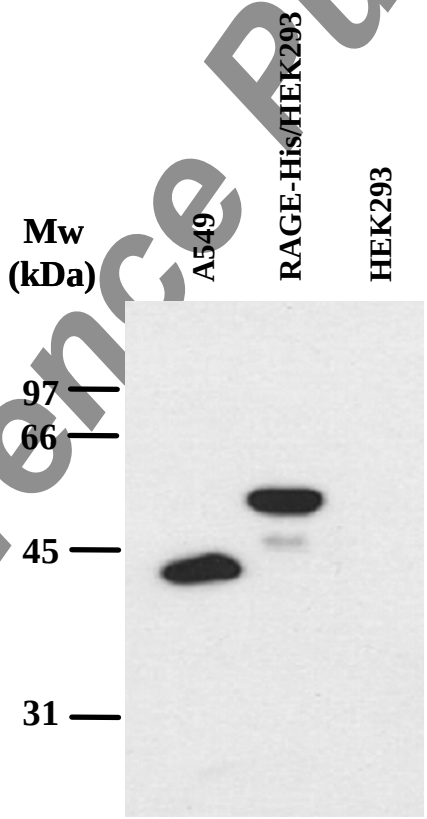


Fig.2 Western blotting analysis of A549 and RAGE-His/HEK293 Cell Line (CY-C8250) by using anti-RAGE monoclonal antibody, KH-2G12 (CY-M1041)



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Western Immunoblotting Protocol

Solutions and Reagents

Note: Prepare solutions with Milli-Q or equivalently purified water.

Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
SDS Sample Buffer (1X): 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
Blocking Buffer: 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 mL, add 15 mL 10X TBS to 135 mL water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 mL Tween-20 (100%).
10X TBS (Tris-buffered saline): To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
Primary Antibody Dilution Buffer: 1X TBS, 0.1% Tween-20 with 5% blocking agent for 20 mL, add 2 mL 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µL Tween-20 (100%).
Chemiluminescent HRP Detection: secondary anti-rabbit antibody conjugated to horseradish peroxidase (HRP), ECL™ chemiluminescent reagent (Amersham Pharmacia)
Wash Buffer TBS/T: 1X TBS, 0.1% Tween-20
Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS, aspirate.
3. Lyse cells by adding 1X SDS Sample Buffer (100 µL per well of 6-well plate or 500 µL per plate of 10 cm2 plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µL sample to 95–100°C for 5 minutes, cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µL onto SDS-PAGE gel (10 cm x 10 cm).
8. Electrotransfer to nitrocellulose membrane.

Membrane Blocking and Antibody Incubations

Note: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 mL TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 mL of Blocking Buffer for 1 hour at room temperature.
3. Wash 3 times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 mL Primary Antibody Dilution Buffer with gentle agitation overnight at 4°C.
5. Wash 3 times for 5 minutes each with 15 mL of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:3000 in 10 mL of Blocking Buffer with gentle agitation for 1 hour at room temperature.
7. Wash 3 times for 5 minutes each with 15 mL of TBS/T.

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Detection of Proteins

1. Incubate membrane with 4 mL ECL™ with gentle agitation for 1 minute at room temperature.
2. Drain membrane of excess developing solution, do not let dry, wrap in plastic wrap and expose to x-ray film. An initial ten-second exposure should indicate the proper exposure time.

Related Products

*** CircuLex Anti-Human RAGE Mouse Monoclonal Antibody (YK-2B4): Cat# CY-M1038**
*** CircuLex Human sRAGE ELISA Kit: Cat# CY-8083**
*** RAGE/HEK293 Cell Line: Cat# CY-C8250**
*** CircuLex Anti-CML / N^{ε}-(Carboxymethyl)lysine (Clone MK-5A10): Cat# CY-M1028**
*** CircuLex AGE-RAGE *in vitro* Binding Assay Kit: Cat# CY-8151**
*** CircuLex S100A12/EN-RAGE ELISA Kit: Cat# CY-8058**

*** CML-BSA / N^{ε}-(Carboxymehtyl)lysine-BSA: Cat# CY-R2052**
*** CML-OVA / N^{ε}-(Carboxymehtyl)lysine-OVA: Cat# CY-R2053**
*** CEL-BSA/ N^{ε}-(Carboxyehtyl)lysine-BSA: Cat# CY-R2054**
*** CEL-OVA/ N^{ε}-(Carboxyehtyl)lysine-OVA: Cat# CY-R2055**
*** Glucose-AGE-BSA: Cat# CY-R2056**
*** Glucose-AGE-OVA: Cat# CY-R2057**
*** Glyceraldehyde-AGE-BSA: Cat# CY-R2058**
*** Glyceraldehyde-AGE-OVA: Cat# CY-R2059**
*** Glycolaldehyde-AGE-BSA: Cat# CY-R2060**
*** Glycolaldehyde-AGE-OVA: Cat# CY-R2061**
*** Methylglyoxal-AGE-BSA: Cat# CY-R2062**
*** Methylglyoxal-AGE-OVA: Cat# CY-R2063**
*** Glyoxal-AGE-BSA: Cat# CY-R2064**
*** Glyoxal-AGE-OVA: Cat# CY-R2065**
*** CML-HSA / N^{ε}-(Carboxymehtyl)lysine-HSA: Cat# CY-R2066**
*** CEL-HSA / N^{ε}-(Carboxyehtyl)lysine-HAS: Cat# CY-R2067**

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