Anti-RAGE (Human) mAb

Cat# CY-M1038

100 µg (1.0 mg/mL x 100 µL)

Clone Name	Applications	Species Cross-Reactivity	Molecular Wt.	Source Isotype
YK-2B4	WB, F	H	46-54 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).

It has been reported that soluble RAGE (sRAGE) isoforms that could act as decoy receptors may be cleaved proteolytically from the native RAGE expressed on the cell surface (14), as well as endogenous secretory RAGE (esRAGE) derived from a splice variant, carrying all of the extracellular domains. This proteolytic generation of sRAGE was initially described as occurring in mice (15). These diverse types of sRAGE in human plasma could exert significant protective effects against RAGE-mediated toxicity.

Specificity/Sensitivity: Anti-RAGE (Human) mAb (YK-2B4) detects overexpressed RAGE by flow cytometory, western blotting and sandwich ELISA.

Source/Purification: Monoclonal 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 blotting, **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 (Species enclosed in parentheses are predicted to react based on 100% sequence homology.)

General References:

1. Neeper M, Schmidt AM, Brett J et al.. J Biol Chem 1992, 267: 14998-5004.

2. Brett J, Schmidt AM, Yan SD et al. Am J Pathol 1993, 143: 1699-712.

3. Neeper M, Schmidt AM, Brett J, et al. J Biol Chem 1992, 267: 14998-15004.

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.

14. Hudson, B.I., Harja, E., Moser, B. and Schmidt, A.M. Arterioscler Thromb Vasc. Biol. 2005, 25: 879–82.

15. Hanford, L.E., Enghild, J.J. et al. J. Biol. Chem. 2004, 279: 50019–24.

Fig.1 Flow cytometory analysis of RAGE/HEK293 Cell Line using Anti-RAGE (Human) mAb (YK-2B4)

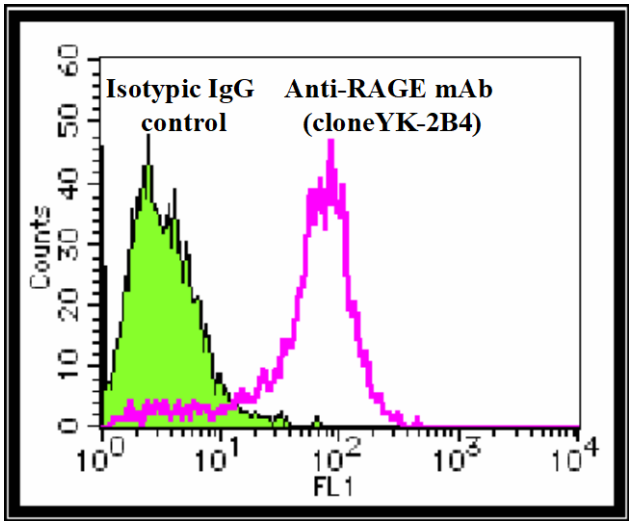
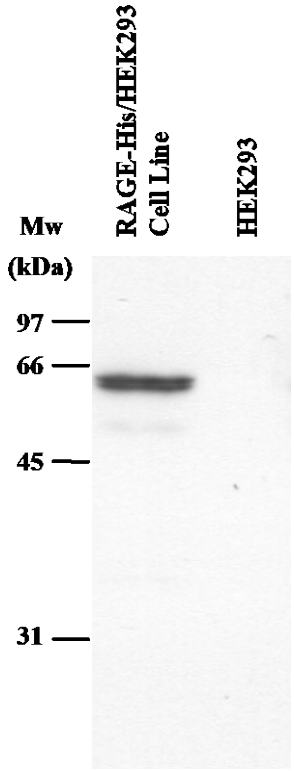


Fig.2 Western blotting analysis of RAGE/HEK293 Cell Line using Anti-RAGE (Human) mAb (YK-2B4)



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-mouse 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.

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.

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