Mouse RAGE Immunoassay

Catalog Number: EA800168

For the quantitative determination of mouse RAGE concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

MANUFACTURED AND DISTRIBUTED BY:

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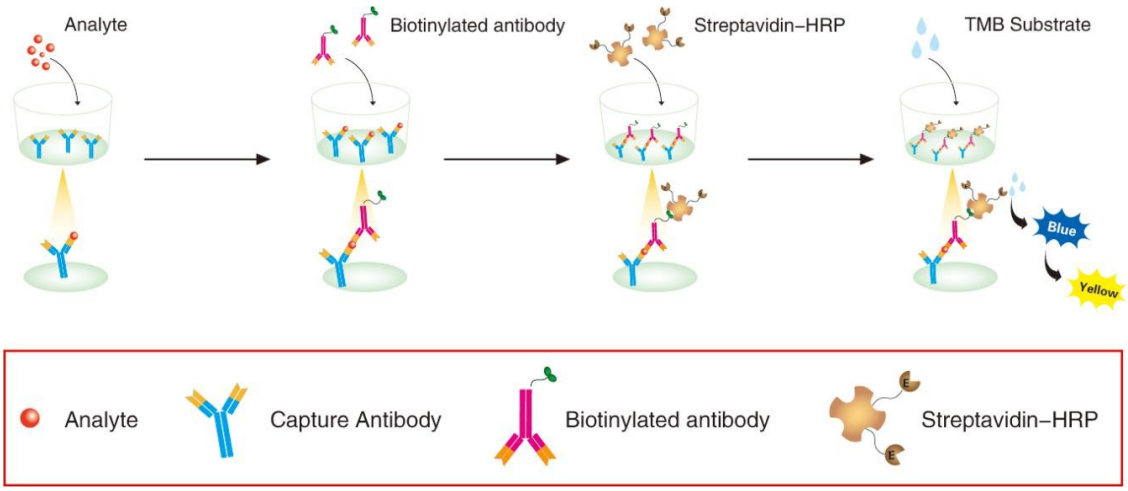
BACKGROUND

Receptor for Advanced Glycation End product (RAGE) is an approximately 50 kDa type I transmembrane glycoprotein belonging to the immunoglobulin (Ig) superfamily. RAGE plays important roles in several pathological processes including inflammation, diabetes, cancer, and Alzheimer's disease (AD). RAGE consists of a 319 amino acid (aa) extracellular domain (ECD) with three Ig-like domains, a 21 aa transmembrane segment, and a 41 aa cytoplasmic domain. Within the ECD, mouse RAGE shares 79% and 91% aa sequence identity with human and rat RAGE, respectively. Alternative splicing of mouse RAGE generates multiple additional isoforms that are truncated at various points within the ECD or carry internal deletions. A soluble form of RAGE (sRAGE) can also be generated by metalloproteinase-mediated cleavage of the ECD. The membrane-bound fragment remaining after ECD shedding can be cleaved by gamma-secretase to release the intracellular domain. RAGE is expressed in the central nervous system (CNS) during development as well as in adult endothelial cells, smooth muscle cells, pericytes, monocytes, and neurons. RAGE binding to S100A1, EN-RAGE/S100A12, or S100B induces inflammatory immune cell adhesion and infiltration as well as vascular smooth muscle proliferation, neointimal expansion, and atherosclerotic plaque development. RAGE also cooperates with TLR9 in the B cell and dendritic cell inflammatory response to complexes of HMGB1 and CpG DNA. In cancer, RAGE binding to HMGB1, S100A8, or S100A9 promotes tumor growth and metastasis in addition to inflammatory cell infiltration. sRAGE functions as a sink for RAGE ligands and lessens the severity of these processes

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for RAGE has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any RAGE present is captured by the coated antibody after incubation. Following extensive washing, a biotin-conjugate antibody specific for RAGE is added to detect the captured RAGE protein in sample. For signal development, horseradish peroxidase (HRP)-conjugated Streptavidin is added, followed by tetramethyl-benzidine (TMB) reagent. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450nm.

Schematic diagram:



TECHNICAL HINTS AND LIMITATIONS

1. This ELISA should not be used beyond the expiration data on the kit label.
2. To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3. To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4. A thorough and consistent wash technique is essential for proper assay performance.
5. A standard curve should be generated for each set of samples assayed.
6. It is recommended that all standards and samples be assayed in duplicate.
7. Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8. In order to ensure the accuracy of the results, the standard curve should be made every time.

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate - antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8℃**
Standard-lyophilized, 4000 pg/ vial upon reconstitution	2 vials	Aliquot and Store at -20°C** for six months
Concentrated Biotin-Conjugated antibody(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
Concentrated Streptavidin-HRP solution(100X) - 120 ul/vial	1 vial	Store at 2-8°C** for six months
Standard /Sample Diluent - 16 ml/vial	1 bottle	Store at 2-8°C** for six months
Biotin-Conjugate antibody Diluent - 16 ml/vial	1 bottle	Store at 2-8°C** for six months
Streptavidin-HRP Diluent - 16 ml/vial	1 bottle	Store at 2-8°C** for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C** for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C** for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

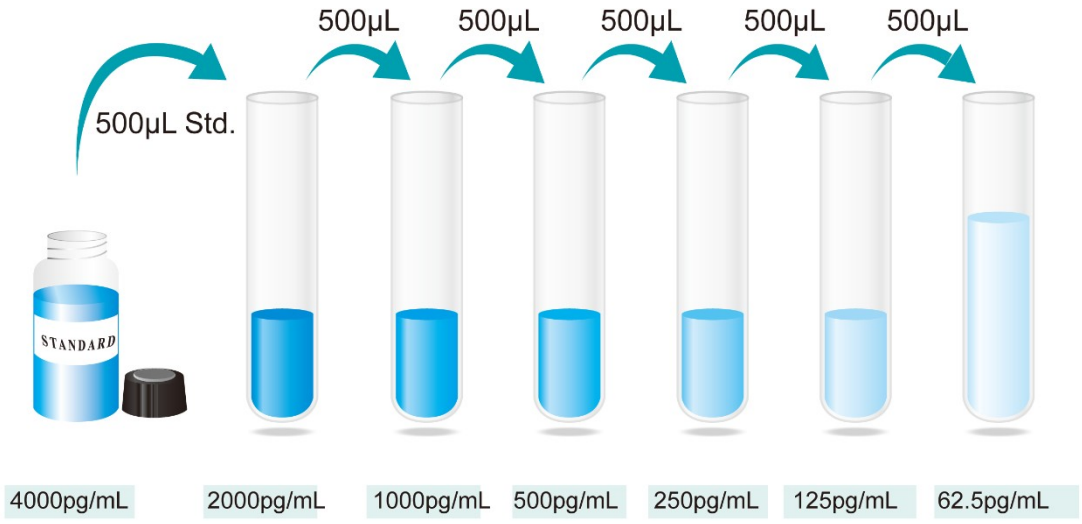
1. Microplate reader capable of measuring absorbance at 450 nm.
2. Pipettes and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.

SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge approximately for 15 minutes at 1000×g. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Note: The normal mouse serum or plasma samples are suggested to make a 1:2 dilution.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25℃) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard/Specimen** - Reconstitute the Standard with 1.0mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 4000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500μL of Standard/ Sample Diluent into 2000pg/ml tube and the remaining tubes. Use the stock solution of 4000pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 4000 pg/mL standard serves as the high standard. The Standard/ Sample Diluent serves as the zero standard (0 pg/mL).



Preparation of RAGE standard dilutions

***If you do not run out of re-melting standard, store it at -20°C. Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-mouse RAGE antibody:** Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

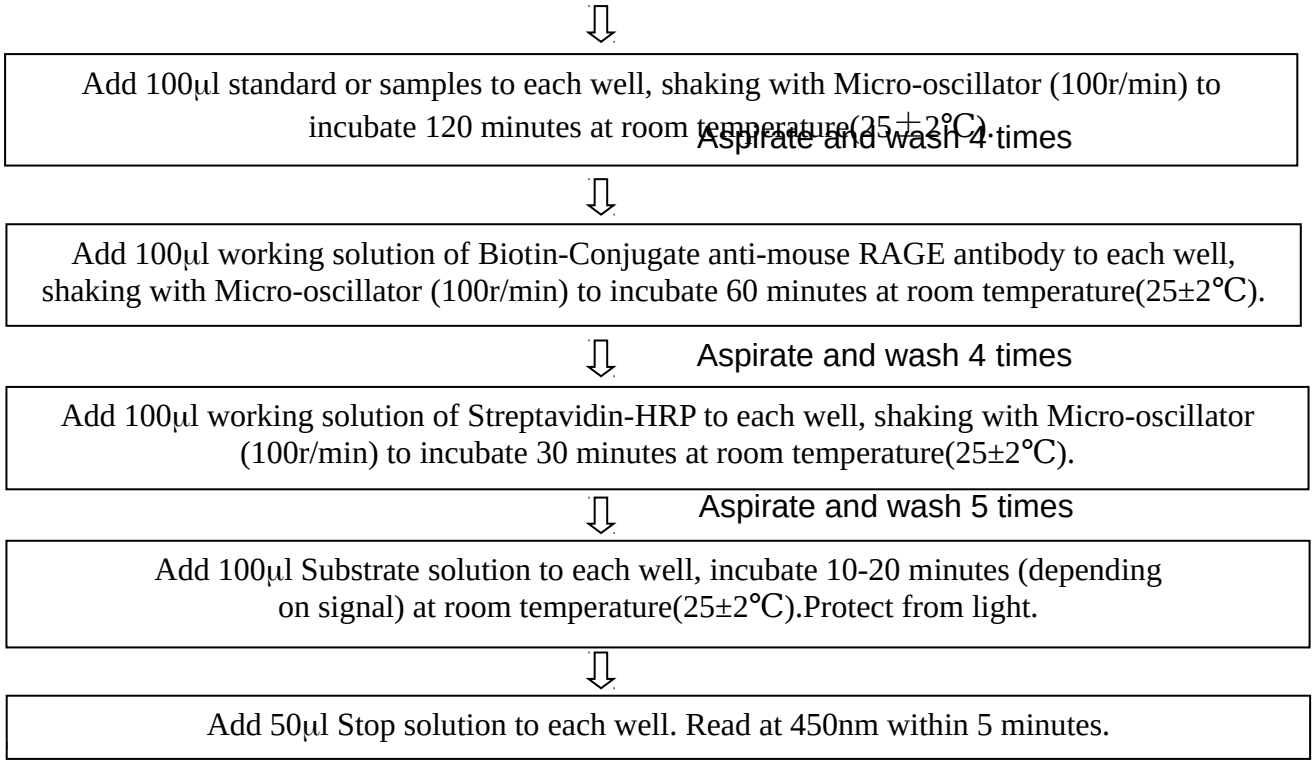
   ***The working solution should be used within one day after dilution.**

5. **Working solution of Streptavidin-HRP**: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.

   ***The working solution should be used within one day after dilution.**

ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

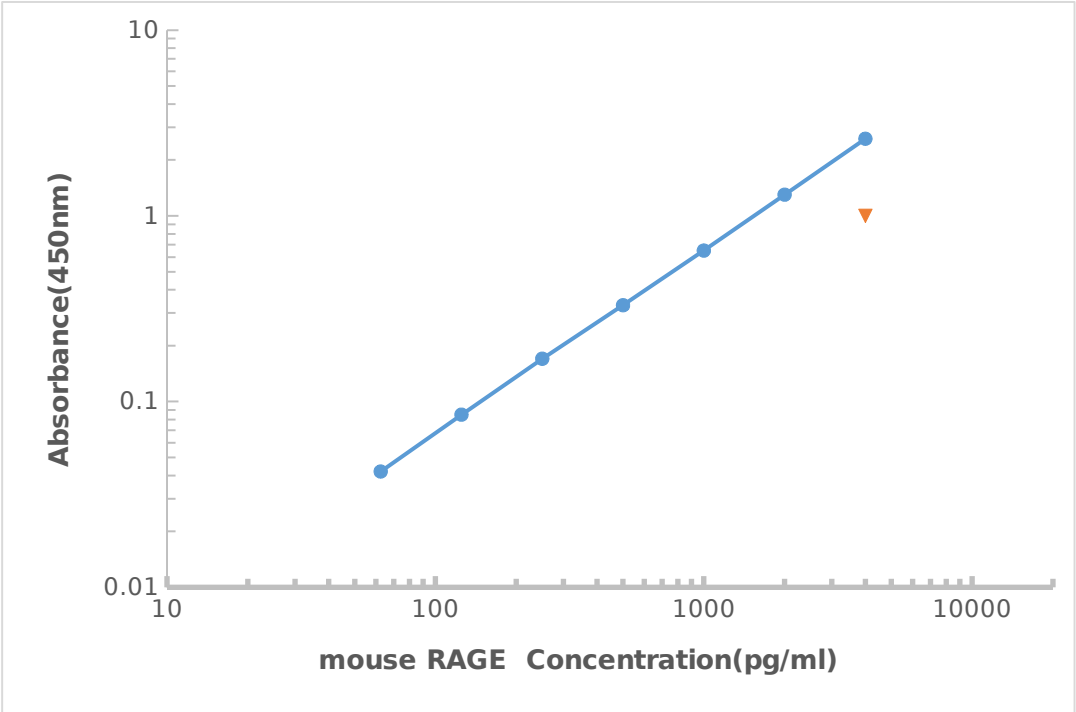


CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the RAGE concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the RAGE ELISA

Standard(pg/ml)	OD.	OD.	Average	Corrected
0	0.018	0.008	0.013	----
62.5	0.206	0.211	0.208	0.195
125	0.238	0.243	0.241	0.228
250	0.354	0.362	0.358	0.345
500	0.562	0.574	0.568	0.555
1000	0.913	0.933	0.923	0.910
2000	1.487	1.518	1.503	1.490
4000	2.423	2.474	2.449	2.435



Representative standard curve for RAGE ELISA.

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 30 pg/mL.

SPECIFICITY: This assay recognizes both natural and recombinant mouse RAGE.

The factors listed below were prepared at 100ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

Recombinant mouse	Recombinant rat	Recombinant human
CD36/SR-B3		Amyloid
Galectin-3		β-Peptide
LOX-1/OLR1		EN-RAGE
S100A10		HMGB1
SR-B1		S100B
Stabilin-2		

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY： The recovery of RAGE spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of RAGE in two matrices

Sample Type	Average % of Expected Range (%)	Range (%)
Citrate plasma	90	82－98
Cell culture supernatants	102	91－112

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of RAGE in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

Dilution ratio	Recovery (%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	93	105
	Range (%)	99－102	95－116
1:4	Average% of Expected	96	107
	Range (%)	88－105	98－117

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