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## Human RAGE Immunoassay

Catalog Number:SEKH-0302

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

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

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**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)

The linearity of the assay

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	101	104
	Range(%)	92–110	96–115
1:4	Average% of Expected	98	103
	Range(%)	89–111	91–114

**Performance Characteristics**

**SENSITIVITY:** The minimum detectable dose was 31 pg/mL

**SPECIFICITY:** A sample containing 50 ng/mL of recombinant human S100A10 was assayed and exhibited no cross-reactivity or interference.

A sample containing 6.25 ng/mL of recombinant mouse RAGE/ Fc Chimera reads as 59 pg/mL (0.9% cross-reactivity).

A sample containing 3.12 ng/mL of recombinant rat RAGE/Fc Chimera reads as 257 pg/mL (8.2% cross-reactivity).

**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	93	86–99
Cell culture supernatants	103	90–108

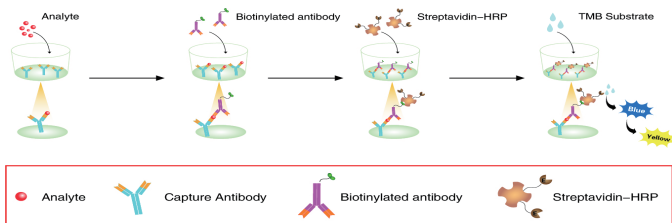
**BACKGROUND**

RAGE (Receptor for Advanced Glycation End product) is a transmembrane glycoprotein that binds advanced glycation end products (AGEs), beta-amyloid peptides, HMGB1/Amphoterin, and several S100 family proteins. AGEs are adducts formed by the non-enzymatic glycation and oxidation of proteins and lipids. A soluble form can also be generated by MMP-mediated shedding. RAGE is expressed in the CNS during development as well as in adult endothelial cells, smooth muscle cells, pericytes, monocytes, and neurons. It is locally upregulated in vascular inflammation (e.g. diabetes, atherosclerosis, vascular injury, Alzheimer's disease). At these sites, 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, atherosclerotic plaque development, and transport of A-beta into the cerebrospinal fluid. In cancer, RAGE binding to HMGB1, S100A8, or S100A9 promotes tumor growth and metastasis in addition to inflammatory cell infiltration.

**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.

## DESCRIPTION



## TECHNICAL HINTS AND LIMITATIONS

1. This Solarbio 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.

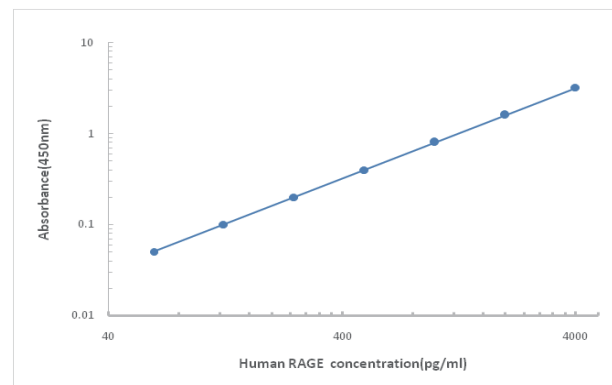
## DESCRIPTION

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

Standardized (pg/ml)	OD.	OD.	Average	Corrected
0	0.048	0.058	0.053	--
62.50	0.173	0.153	0.163	0.110
125.0	0.251	0.222	0.237	0.184
250	0.373	0.330	0.352	0.299
500	0.593	0.525	0.559	0.506
1000	0.963	0.852	0.907	0.855
2000	1.567	1.387	1.477	1.424
4000	2.554	2.260	2.407	2.354



Representative standard curve for RAGE ELISA.

**ASSAY PROCEDURE**

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



Add 100µl standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 120 minutes at room temperature(25±2°C).



Aspirate and wash 4 times

Add 100µl working solution of Biotin-Conjugate anti-human RAGE antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2°C).



Aspirate and wash 4 times

Add 100µl working solution of Biotin-Conjugate anti-duck IgG antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2°C).



Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 10-30 minutes (depending on signal) at room temperature(25±2°C). Protect from light.



Add 50µl Stop solution to each well. Read at 450nm within 5 minutes.

**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

**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°C**
Standard - lyophilized, 4000 pg/ml 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. Squirrt bottle, manifold dispenser, or automated microplate washer.
5. 500 mL graduated cylinder.

**SPECIMEN COLLECTION & STORAGE**

**Cell Culture Supernates** - Centrifuge cell culture media at 1000g (or 3000rpm) to remove debris. Assay immediately or aliquot and store samples at  $\leq -20^{\circ}\text{C}$ . 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  $2-8^{\circ}\text{C}$ . Centrifuge approximately for 15 minutes at 1000g (or 3000rpm). Assay immediately or aliquot and store samples at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000g (or 3000rpm) within 30 minutes of collection. Assay immediately or aliquot and store samples at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.

**Note:** It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

**REAGENTS PREPARATION**

1. **Temperature returning** - Bring all kit components and specimen to room temperature ( $20-25^{\circ}\text{C}$ ) 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\Sample** - Reconstitute the Standard with 0.5mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 4000pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 $\mu\text{L}$  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 4000pg/mL standard serves as the high standard. The Standard/ Sample Diluent serves as the zero standard (0 pg/mL).

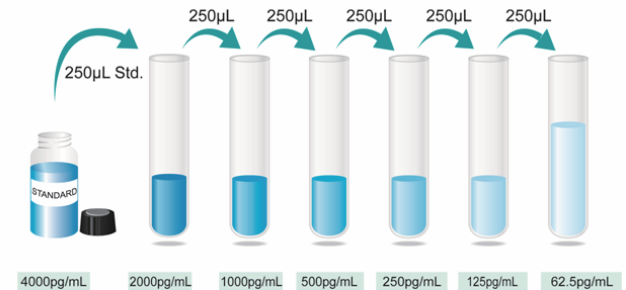
**\*If you do not run out of re-melting standard, store it at  $-20^{\circ}\text{C}$ . Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-human 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.**



Preparation of RAGE standard dilutions