Rat RAGE ELISA Kit

Enzyme Immunoassay for the quantification of Rat RAGE in Serum, plasma (heparin, EDTA) and cell culture supernatants.

Catalog number: ARG81791

Package: 96 wells

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INTRODUCTION

The advanced glycosylation end product (AGE) receptor encoded by this gene is a member of the immunoglobulin superfamily of cell surface receptors. It is a multiligand receptor, and besides AGE, interacts with other molecules implicated in homeostasis, development, and inflammation, and certain diseases, such as diabetes and Alzheimer's disease. Many alternatively spliced transcript variants encoding different isoforms, as well as non-protein-coding variants, have been described for this gene (PMID:18089847). [provided by RefSeq, May 2011]

Mediates interactions of advanced glycosylation end products (AGE). These are nonenzymatically glycosylated proteins which accumulate in vascular tissue in aging and at an accelerated rate in diabetes. Acts as a mediator of both acute and chronic vascular inflammation in conditions such as atherosclerosis and in particular as a complication of diabetes. AGE/RAGE signaling plays an important role in regulating the production/expression of TNF-alpha, oxidative stress, and endothelial dysfunction in type 2 diabetes. Interaction with S100A12 on endothelium, mononuclear phagocytes, and lymphocytes triggers cellular activation, with generation of key proinflammatory mediators. Interaction with S100B after myocardial infarction may play a role in myocyte apoptosis by activating ERK1/2 and p53/TP53 signaling (By similarity). Receptor for amyloid beta peptide. Contributes to the translocation of amyloid-beta peptide (ABPP) across the cell membrane from the extracellular to the intracellular space in cortical neurons. ABPP-initiated RAGE signaling, especially stimulation of p38 mitogen-activated protein kinase (MAPK), has the capacity to drive a transport system delivering ABPP as a complex with RAGE to the intraneuronal space. Can also bind oligonucleotides. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for RAGE has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any RAGE present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for RAGE is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of RAGE bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of RAGE in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

NO	Component	Quantity	Storage information
C1	Antibody-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
C2	Standard (Lyophilized)	2 X 10 ng/vials	4°C
C3	Standard/Sample diluent buffer	30 ml (ready to use)	4°C
C4	Antibody conjugate concentrate (100X)	1 vial (100 µl)	4°C
C5	Antibody diluent buffer	12 ml (ready to use)	4°C
C6	HRP-Streptavidin concentrate (100X)	1 vial (100 µl)	4°C (Protect from light)
C7	HRP-Streptavidin diluent buffer	12 ml (ready to use)	4°C
C8	25X Wash buffer	20 ml	4°C
C9	TMB substrate	10 ml (ready to use)	4°C (Protect from light)
C10	STOP solution	10 ml (ready to use)	4°C
C11	Plate sealer	4 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All materials should be equilibrated to room temperature (RT, 22-25°C) 20 min before use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Mix the contents of the microplate wells thoroughly by microplate shaker for 1 min or gently tap the plate to ensure good test results. Please mix carefully to avoid well-to-well contamination. Do not reuse microwells.
- The TMB Color developing agent should be colorless and transparent before using.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.

- Avoid using reagents from different batches.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation for 10 min at 1500 x g at 4°C and aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

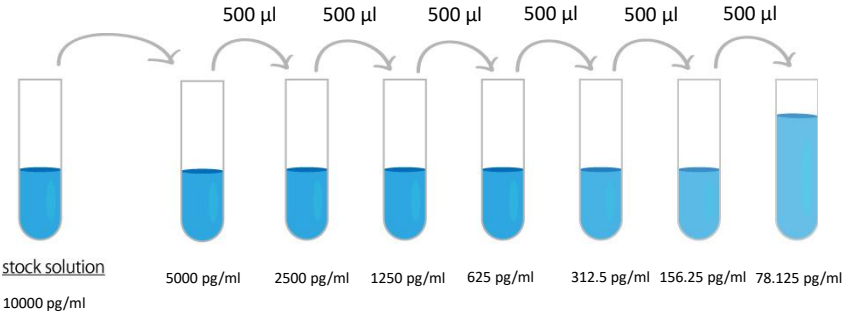
Note:

a) Do not use haemolytic, icteric or lipaemic specimens.

b) Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 25X Wash buffer (#C8) into distilled water to yield 1X Wash buffer. (E.g. 40 ml of 25X Wash buffer + 960 ml of distilled water) The diluted Wash buffer is stable for 4 weeks at 2°C to 8°C.
- 1X Antibody conjugate: 20 minutes before use, dilute 100X antibody conjugate concentrate (#C4) into antibody diluent buffer (#C5) to yield 1X Detection antibody solution.
- 1X HRP-Streptavidin Solution: 20 minutes before use, dilute 100X HRP-Streptavidin concentrate solution (#C6) into HRP-Streptavidin diluent buffer (#C7) to yield 1X HRP-Streptavidin Solution buffer. Keep diluted HRP-Streptavidin Solution in dark before use.
- Sample: If the initial assay found samples contain RAGE higher than the highest standard, the samples can be diluted with Standard diluent buffer (#C3) and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.
  (It is recommended to do pre-test to determine the suitable dilution factor).
- Standards: Reconstitute the standard (#C2) with 1 ml standard diluent buffer (#C3) to yield a stock concentration of 10000 pg/ml. Keep the buffer in the vail for at least 15 min at RT to make sure the standard is dissolved completely before making serial dilutions. The standard diluent buffer serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: 5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 312.5 pg/ml, 156.25 pg/ml, 78.125 pg/ml. DO NOT reuse the reconstituted standard.



Dilute RAGE standard as according to the table below:

Standard	RAGE Conc.	µl of Standard diluent	µl of standard
S7	5000 pg/ml	500	500 (10000 pg/ml Stock)
S6	2500 pg/ml	500	500 (S7)
S5	1250 pg/ml	500	500 (S6)
S4	625 pg/ml	500	500 (S5)
S3	312.5 pg/ml	500	500 (S4)
S2	156.25 pg/ml	500	500 (S3)
S1	78.125 pg/ml	500	500 (S2)
S0	0	500	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) or 37°C before use. The 1X HRP-Streptavidin Solution and TMB substrate must be kept warm at 37°C before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard RAGE detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of RAGE amount in samples. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **100 µl** of standards, samples and zero controls (S0, Standard/Sample diluent) into wells. Cover the plate and incubate for **90 minutes at 37°C**.
3. Aspirate each well. Complete removal of liquid by aspirating, decanting or blotting against clean paper towels. DO NOT let the wells completely dry at any time. Wash step is not necessary in this step.
4. Add **100 µl** 1X Antibody conjugate into each well, gently tap the plate to mix well. Cover wells and incubate for **60 minutes at 37°C**.
5. Aspirate each well and wash, repeating the process two times for a total **three washes**. Wash by filling each well with 1X Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher, keep the wash buffer in the wells for 1 min before remove. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels. DO NOT let the wells completely dry at any time.

6. Add 100 µl of 1X HRP-Streptavidin solution to each well, gently tap the plate to mix well. Cover wells and incubate for 30 minutes at 37°C.
7. Aspirate each well and wash as step 5.
8. Add 90 µl of TMB substrate to each well. Incubate for 20-25 minutes at 37°C in dark. (Note: The incubation time is for reference only, the optimal incubation time should be determined by end user. And the shades of blue color can be seen in the wells with the four most concentrated RAGE standard solutions; the other wells show no obvious color).
9. Add 100 µl of Stop Solution to each well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough mixing
10. Read the OD with a microplate reader at 450nm immediately. It is recommended read the absorbance within 10 minutes after adding the stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

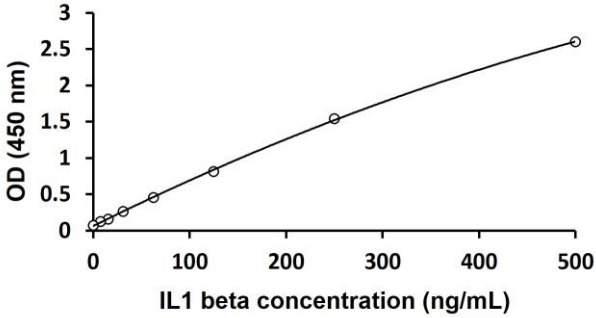
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Rat RAGE ranged from 78 pg/ml- 5000 pg/ml. The mean MDD was 39 pg/ml.

Specificity

This assay recognizes natural and recombinant Rat RAGE.

There is no detectable cross-reactivity with other relevant proteins.

Intra-assay and Inter-assay precision

The CV values of both intra and inter precision fall below 10%.