Human RAGE ELISA Kit

Enzyme Immunoassay kit for the quantification of Human RAGE (AGER) in Human serum, plasma and cell culture supernatants samples.

Catalog number: ARG81343

Package: 96 wells

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan (R.O.C.)

Phone: +886 (3) 621 8100

Fax: +886 (3) 553 0266

Email: info@arigobio.com

INTRODUCTION

RAGE (advanced glycosylation end product (AGE) receptor) is a member of the immunoglobulin superfamily of cell surface receptors. It is a multi-ligand 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. [provided by RefSeq, May 2011]

RAGE / AGER mediates interactions of advanced glycosylation end products (AGE). These are non-enzymatically 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 pro-inflammatory mediators. Interaction with S100B after myocardial infarction may play a role in myocyte apoptosis by activating ERK1/2 and p53/TP53 signaling. 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 sandwich enzyme immunoassay technique for the detection of Human RAGE in plasma and cell culture supernatants samples. 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 on the plate. After washing away any unbound substances, a Horseradish Peroxidase (HRP) conjugated primary antibody binds to RAGE is added to each well and incubates. 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 total 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 total RAGE in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

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

Component	Quantity	Storage information
antibody-coated microplate	12 X 8 strips	4°C
Standard	1 vial	4°C, store at -20 °C after reconstitution
Standard reconstitution buffer	1.1 ml (ready-to-use)	4°C
HRP-antibody Conjugate (100X)	115 µl	4°C
Sample & Antibody diluent buffer	20 ml (ready-to-use)	4°C

Assay Buffer	6 ml (ready-to-use)	4°C
10X Wash buffer	20 ml	4°C
TMB substrate	12 ml (ready-to-use)	4°C (Protect from light)
STOP solution	6 ml	4°C
Plate sealer	1 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- 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 4°C at all times.
- Briefly spin down the HRP-antibody conjugate before use.
- If crystals are observed in the 10X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved. 1X Wash buffer should be prepared and stored at 4°C before use.
- Ensure complete reconstitution and dilution of reagents prior to use.
- 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.
- Samples contain azide cannot be assayed.

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 and aliquot & store samples at ≤ -20 °C. 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. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. 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. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- Standard: Reconstitute the standard with 0.35 ml Standard reconstitution buffer to yield a stock concentration of 25 ng/ml. Vortex the tube for 1 min and stand for 10 minutes to completely dissolve contents. Aliquot and store at ≤ -20°C for long term storage.

  Make sure the standard is dissolved completely before making serial dilutions. The Sample & Antibody diluent buffer serves as zero standard (0 pg/ml), and the standard stock can be diluted with Sample & Antibody diluent buffer 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 and 78.125 pg/ml.

Dilute RAGE standard as according to the table below:

Standard	RAGE Conc. (pg/ml)	µl of Sample & Antibody diluent buffer	µl of standard
S7	5000	400	100 (25 ng/ml Stock)
S6	2500	200	200 (S7)
S5	1250	200	200 (S6)
S4	625	200	200 (S5)
S3	312.5	200	200 (S4)
S2	156.25	200	200 (S3)
S1	78.125	200	200 (S2)
S0	0	200	0

- **1X Wash buffer**: Dilute 10X wash buffer with distilled water to yield 1X wash buffer. The diluted Wash buffer should be stored at 4 °C.
- **HRP-antibody Conjugate:** Diluent 100X HRP-antibody Conjugate into Sample & Antibody diluent buffer to yield 1X Detection antibody solution. The 1X HRP-antibody Conjugate solution should be used immediately.
- **Sample:** If the initial assay found samples contain RAGE higher than the highest standard, the samples can be diluted with Sample & Antibody diluent buffer and re-assay the samples.

  For the calculation of the concentrations this dilution factor has to be taken into account.

  Example:

  Dilution 1:10: 10 µl sample + 90 µl Sample & Antibody diluent buffer (mix thoroughly).

ASSAY PROCEDURE

All materials do not need equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

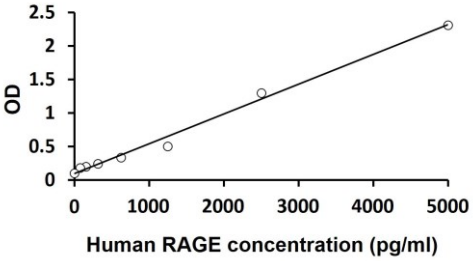
1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 50 µl of standards, samples and zero controls into appropriate wells.
3. Add 50 µl of Assay buffer into all wells immediately. Mix thoroughly by gently shaking or tapping the plate. Cover the plate and incubate for 2h at 37°C.
4. Aspirate each well and wash, repeating the process two times for a total three washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. 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.
5. Add 100 µl 1X HRP-antibody conjugate into each well. Mix thoroughly by gently shaking or tapping the plate. Cover wells and incubate for 1 hour at 37°C.
6. Aspirate each well and wash as step 4.
7. Add 100 µl of TMB substrate to each well. Incubate for 10 minutes at RT in dark. Substrate will change from colorless to different strengths of blue.
8. Add 50 µl of Stop solution to each well. The color of the solution should change from blue to yellow. Mix thoroughly by gently shaking the plate.
9. Read the OD with a microplate reader at 450 nm immediately.

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. 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

Standard range: 78.125 – 5000 pg/ml

Minimum Detectable Concentration: 39 pg/ml

Precision:

Intra-assay: 4.2%

Inter-assay: 6.8%

Recovery:

90.5-101.1%

Specificity

This assay recognizes natural and recombinant total human RAGE.