HUMAN SOLUBLE RECEPTOR FOR ADVANCED GLYCOSYLATION END PRODUCTS (sRAGE) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN sRAGE CONCENTRATIONS IN SERUM, EDTA PLASMA

PURCHASE INFORMATION:

ELISA NAME	HUMAN sRAGE ELISA
Catalog No.	SK00112-01
Lot No.	
Formulation	96 T
Standard range	39 – 5000 pg/mL
Sensitivity	19 pg/mL
Sample Volume	100 µl
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application.
Sample Type	Serum, EDTA plasma
Specificity	Human sRAGE
Intra-assay Precision	4%
Inter-assay Precision	8%
Storage	2-8 °C

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INTRODUCTION

Human soluble RAGE immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human sRAGE in serum and EDTA plasma. It contains recombinant human sRAGE and antibodies raised against this protein. It has been shown to accurately quantify recombinant human sRAGE. Results obtained with naturally occurring sRAGE samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural Human sRAGE.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for sRAGE has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any sRAGE present is bound by the immobilized antibody. After washing away any unbound substances, a polyclonal antibody specific for sRAGE is added to the wells. Following a wash to remove any unbound antibody, Anti Rabbit IgG-HRP conjugate is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of sRAGE bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute reagents with those from other lots or sources.

_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_ If samples generate values higher than the highest standard, dilute the samples with the appropriate Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Description	Code	Quantity
sRAGE Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal purified IgG against Human sRAGE.	**112-01-01**	**1 plate**
sRAGE Standard – 10,000 pg/vial of recombinant Human sRAGE in a buffered protein base with preservatives; lyophilized.	**112-01-02**	**1 vial**
Detection Antibody Concentrate – 105 µL/vial, 100-fold concentrated of polyclonal purified IgG against Human sRAGE with preservatives; lyophilized.	**112-01-03**	**1 vial**
Positive Control – one vial of recombinant Human sRAGE , lyophilized	**112-01-04**	**1 vial**
Anti Rabbit IgG-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of ARIG-HRP conjugate with preservatives	**ARIGHRP**	**1 vial**
Dilution Buffer - 60mL of buffered protein based solution with preservatives	**DB06**	**1 bottle**
HRP Diluent Solution – 12mL of buffered protein based solution with preservatives	**DB08**	**1 bottle**
Wash Buffer - 50 ml of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution-11 ml of TMB substrate solution	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.5M HCl	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 12 months. For longer storage, unopened Standard, Positive Control and Antibody Concentrate should be stored at -20 or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard, Positive Control and Antibody Concentrate SHOULD BE STORED at -20 °C or -70°C for up to one month. ARIG-HRP Conjugate 100-fold concentrate and other components may be stored at 2 - 8°C for up to 12 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack, reseal along the entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8° C after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

SAMPLE COLLECTION AND STORAGE

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 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.
Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

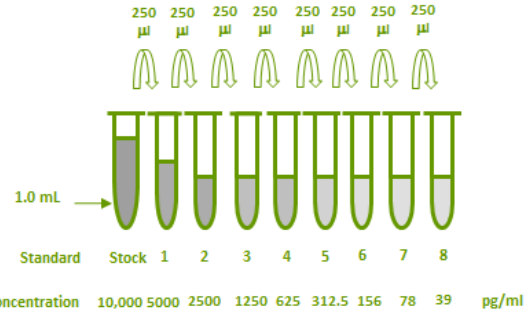
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.

sRAGE Standard - Refer to vial label for reconstitution volume. Reconstitute the **sRAGE** Standard with 1.0 ml of Dilution Buffer. This reconstitution produces a stock solution of 10,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of the appropriate Dilution Buffer into the tube #1 to #8. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 5000 pg/mL standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 pg/mL).

Tube	Standard	Dilution Buffer	Concentration
stock	powder	1 ml	10,000 pg/ml
# 1	250µl of stock	250µl	5000 pg/ml
# 2	250µl of 1	250µl	2500 pg/ml
# 3	250µl of 2	250µl	1250 pg/ml
# 4	250µl of 3	250µl	625 pg/ml
# 5	250µl of 4	250µl	312.5 pg/ml
# 6	250µl of 5	250µl	156 pg/ml
# 7	250µl of 6	250µl	78 pg/ml
# 8	250µl of 7	250µl	39 pg/ml



Detection Antibody Concentrate - Reconstitute the Detection Antibody concentrate with 105 µl of Dilution Buffer to produce a 100-fold concentrated stock solution. Pipette 10.395 mL of the appropriate Dilution Buffer into the 15 ml centrifuge tube and transfer 105 µl of 100-fold concentrated stock solution to prepare working solution.

Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer. **Note:** Positive Control should be prepared and used immediately.

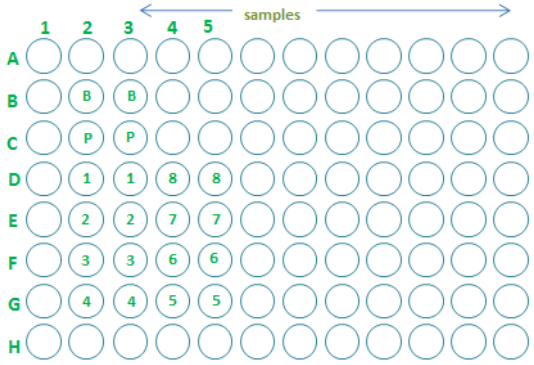
Anti Rabbit IgG-HRP Conjugate - Pipette 11.88 mL of Dilution Buffer into the 15 ml centrifuge tube and

transfer 120 µl of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of ARIG-HRP conjugate should be used within a few days.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack, reseal.
3. Add 100 µL of Dilution Buffer to Blank well (B2, B3).
4. Add 100 µL of Standard (from D2, D3 to G2, G3 and D4, D5 to G4, G5), sample, or positive control (C2, C3) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Detection Antibody working solution to each well. Cover with sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of ARIG-HRP conjugate working solution to each well. Incubate for 1 hour on micro-plate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 4-6 minutes at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log 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. The data may be linearized by plotting the log of the sRAGE 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.

Calculation of samples with a concentration exceeding that of 5000pg/mL may result in inaccurate, low human sRAGE levels. Such samples require further external predilution according to expected human sRAGE values with Dilution Buffer in order to precisely quantify the actual human sRAGE level.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. Coli*-expressed recombinant human sRAGE.

TYPICAL DATA

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)*
Blank	0 (0.129)
39	0.016
78	0.037
156	0.077
312.5	0.138
625	0.293
1250	0.655
2500	1.313
5000	2.340

- **Lot No.:**
- **Positive Control: 600-1300 pg/mL**

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of sRAGE was 19 pg/mL.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Human sRAGE	100
Mouse sRAGE	0
Rat sRAGE	0
Human S100A6	0
Human Flt1	0

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4. Krechler T, et al. Soluble receptor for advanced glycation end-products (sRAGE) and polymorphisms of RAGE and glyoxalase I genes in patients with pancreas cancer. Clin Biochem. 2010 Jul;43(10-11):882-6. Epub 2010 Apr 14.

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⬇
Add 100 µl of standard, samples, positive control each well. Incubate 2 hours on the plate shake at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Detection Antibody Working Solution to each well. Incubate 2 hours on the plate shaker at RT.
⬇
Add 100 µl ARIG-HRP conjugate working solution to all wells. Incubate 60 min on the plate shaker at RT. **Protect from light.**
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Substrate Solution to each well. Incubate 4-6 min on plate shaker. **Protect from light.**
⬇
Add 100 µl Stop Solution to each well. Read 450nm within 15 min