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

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

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PURCHASE INFORMATION:

ELISA NAME	HUMAN sRAGE ELISA
Catalog No.	SK00112-01
Lot No.	
Formulation	96 T
Standard range	78 – 10,000 pg/mL
Sensitivity	50 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 - 8%
Inter-assay Precision	8 - 12%
Storage	2 - 8° C

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INTRODUCTION

Human soluble RAGE immunoassay is a 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

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

_ 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 preservative; lyophilized.	**112-01-02**	**1 vial**
Detection Antibody Concentrate – 1.05 mL/vial, 10-fold concentrate of polyclonal purified IgG against human sRAGE with preservative; 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 preservative.	**ARIGHRP**	**1 vial**
Dilution Buffer – 60 mL of buffered protein based solution with preservative.	**DB06**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**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 8 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20° C or -70° C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard (stock) and Detection Antibody concentrated solution SHOULD BE STORED at -20° C

or -70° C for up to one month. ARIG-HRP Conjugate 100-fold concentrated solution (**protect from light**) and other components may be stored at 2 - 8° C for up to 8 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack and seal 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.
- 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.
Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

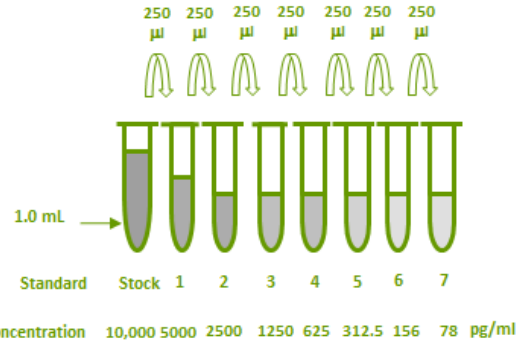
SAMPLE PREPARATION

Plasma and serum samples may not need to be diluted. **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 1x 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 tubes #1 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **10,000 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



Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.05 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Pipette 9.45 mL of the appropriate Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer. **Note:** Positive Control could be reused within a few days if stored at -20° C or -70° C.

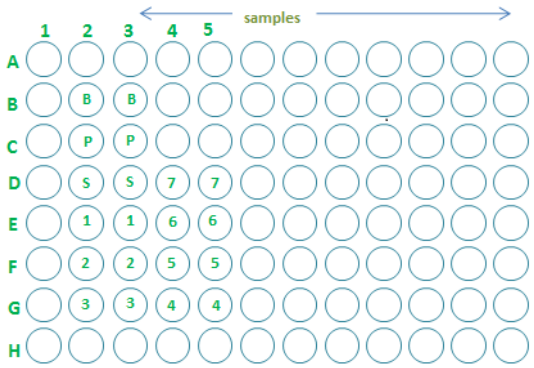
Anti Rabbit IgG-HRP Conjugate - Pipette 11.88 mL of **HRP Diluent Solution (DB08)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. **Note:**

1x working solution of ARIG-HRP conjugate (**protect from light**) 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 duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL of Dilution Buffer to Blank wells (B2, B3).
4. Add 100 µL of Standard solutions in reverse order of serial dilution (from D4, D5 to G4, G5 and G2, G3 to D2, D3), sample, or positive control (C2, C3) per well. Cover with plate sealer. Incubate for 2 hours on microplate 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 1x 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 plate sealer. Incubate for 2 hours on microplate 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 microplate 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 3-8 minutes on microplate shaker 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 microplate 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 10,000 pg/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 recombinant human sRAGE.

SENSITIVITY

The minimum detectable dose (MDD) of sRAGE was 50 pg/mL.

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 (0.130)
78	0.07
156	0.011
312.5	0.025
625	0.069
1250	0.189
2500	0.498
5000	1.098
10000	2.033

- **Lot No.:**
- **Positive Control:**
-

SPECIFICITY

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

REFERENCES:

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2. Yamagishi S, Matsui T. Soluble form of a receptor for advanced glycation end products (sRAGE) as a biomarker. Front Biosci (Elite Ed). 2010 Jun 1;2:1184-95.
3. Shang L, et al. RAGE modulates hypoxia/reoxygenation injury in adult murine cardiomyocytes via JNK and GSK-3beta signaling pathways. PLoS One. 2010 Apr 9;5(4):e10092.
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 shaker 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 each well. 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 3-8 min on plate shaker at RT. **Protect from light.**
↓
Add 100 µL Stop Solution to each well. Read 450nm within 15 min.