

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

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



ALWAYS REFER TO LOT SPECIFIC
PROTOCOL PROVIDED WITH EACH KIT FOR
INSTRUCTIONS. PROTOCOL MUST BE
READ BEFORE USING THIS PRODUCT.

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

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	HUMAN sRAGE ELISA
Catalog No.	SK00112-01
Lot No.	20114599
Formulation	96 T
Standard range	156 – 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
Calibration	Human sRAGE recombinant
Intra-assay Precision	4 - 8%
Inter-assay Precision	4 - 9%
Storage	2 - 8° C for 4 months. See page 2 for detail
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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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

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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.

COMPONENTS 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 mL/vial, 32-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
High Sensitivity Anti Rabbit IgG-HRP Conjugate - 120 µl/vial, 100-fold concentrated solution of ARIG-HRP conjugate with preservative.	HSARIGHRP	1 vial
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	DB06	1 bottle
Antibody Diluent Solution – 25 mL of buffered protein based solution with preservative.	DB36	1 bottle
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	DB08C	1 bottle
Wash Buffer 20X - 25 mL of 20-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 0.25M HCl.	S-STOP	1 bottle
Plate Sealer	EAPS	1
Plastic Pouch	P01	1

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 4 months. For longer storage for up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and HRP Diluent Solution should be stored at -20° C. Anti Rabbit IgG-HRP Conjugate and TMB Substrate Solution should be stored only at 2 -8 °C. Do not use kit past expiration date.

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.

SAMPLE PREPARATION

Plasma and serum samples DO NOT need to be diluted. **Optimal dilutions should be determined by each laboratory for each application.** Use polypropylene test tubes.

REAGENT PREPARATION

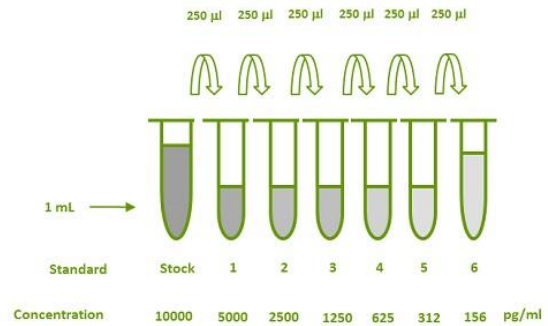
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 25 mL of Wash Buffer Concentrate 20X into deionized or distilled water (475 mL) to prepare 500 mL of 1x Wash Buffer.

sRAGE Standard - 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 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 Dilution Buffer serves as the zero standard (0 pg/mL). Store the stock solution at -70 °C for a few days.

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



Positive Control - Reconstitute the Positive Control with 1 mL of Dilution Buffer.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1 mL of **Antibody Diluent Solution (DB36)** to produce a 32-fold concentrated stock solution. For the 96 wells test freshly Pipette 11.625 mL of **Antibody Diluent Solution (DB36)** into a 15 mL centrifuge tube and transfer 0.375 mL of 32-fold concentrated stock solution to prepare working solution. For the partial strip test, freshly prepare 900 µL per strip of working solution. Store the stock solution (32-fold concentrated) at -20 °C for a few days.

High Sensitivity Anti Rabbit IgG-HRP Conjugate - For the 96 wells test, freshly 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 10-20 min.

For the partial strip test, freshly prepare 900 µL per strip of working solution. Store the stock solution (100-fold concentrated) at 2 ~ 8 °C for 10 months.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 µL of Dilution Buffer to Blank wells.
3. Add 100 µL of standard dilutions in reverse order of serial dilution, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
4. 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.
5. 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.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of HSARIGHRP conjugate working solution to each well. Incubate for 1 hour on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of Substrate Solution to each well. Incubate for 20 minutes on microplate shaker at room temperature. **Protect from light.**
10. 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 2 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.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

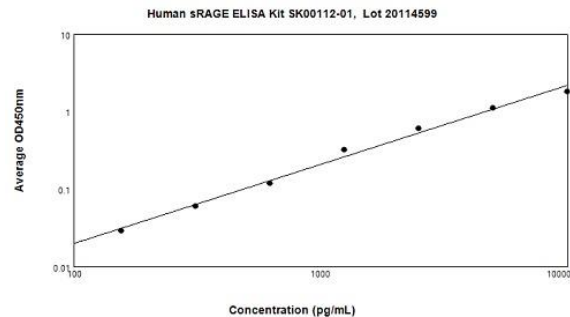
Calculation of samples with a concentration exceeding that of 10,000 pg/mL may result in inaccurate, low human sRAGE levels. Such samples require further external predilution according to expected human sRAGE values with Dilution Buffer in order to precisely quantify the actual human sRAGE level.

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 OD450NM (CORRECTED)*
Blank	0 (0.116)
156	0.029
312.5	0.060
625	0.119
1250	0.319
2500	0.608
5000	1.098
10000	1.784

- Lot No.: 20114599
- Positive Control: 750 - 1430 pg/mL (log-log)



SPECIFICITY

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

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 μ L of standard dilutions, samples, or positive control each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 μ L Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 μ L 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 μ L Substrate Solution to each well. Incubate 20 min on plate shaker at RT. Protect from light.
↓
Add 100 μ L Stop Solution to each well. Read 450nm within 2 min.