MOUSE SOLUBLE RAGE ELISA KIT

For the quantitative determination of Mouse sRAGE concentrations in serum, EDTA Plasma

PURCHASE INFORMATION:

ELISA NAME	MOUSE SRAGE ELISA
Catalog No.	SK00112-04
Lot No.	
Formulation	96 T
Standard range	62.5 – 4000 pg/mL
Sensitivity	20 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	Mouse sRAGE
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2°C - 8°C

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

Order Contact:
AVISCERA BIOSCIENCE INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: Sales@AvisceraBioscience.com
Info@AvisceraBioscience.com
www.AvisceraBioscience.com

INTRODUCTION

Mouse soluble RAGE immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure mouse sRAGE in serum and EDTA plasma. It contains recombinant mouse sRAGE and antibodies raised against this protein. It has been shown to accurately quantify recombinant Mouse 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 Mouse 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. The addition of Sample Buffer to all the wells helps reduce the non-specific binding of mouse sample and standards to the plate. Some samples may be read close to the background level. 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 biotinylated polyclonal antibody specific for sRAGE is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is add 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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_ Some vials contain small quantities of material, therefore centrifuge before use.

_ 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 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
Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal purified IgG against Mouse sRAGE.	112-04-01	1 plate
sRAGE Standard – 4000 pg/vial of recombinant Mouse sRAGE in a buffered protein base with preservatives; lyophilized.	112-04-02	1 vial
Detection Antibody Concentrate – 105 µL/vial, 100-fold concentrated of Biotinylated polyclonal purified IgG against Mouse sRAGE with preservatives; lyophilized.	112-04-03	1 vial
Positive Control – one vial of recombinant Mouse sRAGE , lyophilized	112-04-04	1 vial
Streptavidin-HRP Conjugate - 75 µL/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP with preservatives	SAHRP	1 vial
Dilution Buffer - 60mL/vial of buffered protein based solution with preservatives	DB18	1 vial
Sample Buffer – 10mL/vial of buffered solution	DB00	1 vial
Wash Buffer - 50 mL/vial, 10-fold concentrated buffered surfactant, with preservative.	WB01	1 vial
TMB Substrate Solution - 11mL/vial of TMB substrate solution	TMB01	1 vial
Stop Solution – 11 mL/vial of 0.5N HCl	S-STOP	1 vial
Plate Sealer	EAPS	1 piece

STORAGE

Unopened Kit: Store at 2 – 8 °C for up to 6 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 or -70 °C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard, Antibody Solution COULD BE STORED at -20°C or -70°C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrated and other components may be stored at 2 - 8°C for up to 6 months. Reconstituted Positive Control should be prepared and used immediately.

Microplate Wells: Return unused wells to the plastic bag containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 6 months at 2 - 8°C.

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

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and 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 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.

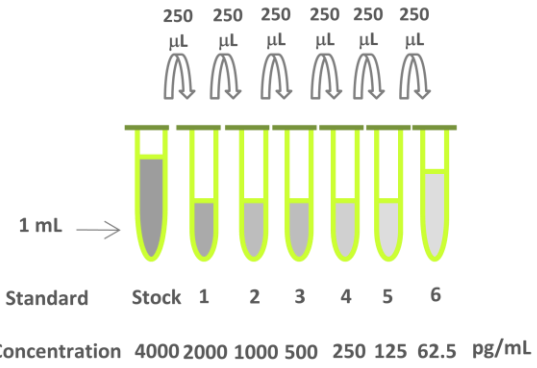
REAGENT PREPARATION

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 mL of Dilution Buffer. This reconstitution produces a stock solution of 4000 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 #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4000 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1 mL	4000 pg/mL
# 1	250µL of stock	250µL	2000 pg/mL
# 2	250µL of 1	250µL	1000 pg/mL
# 3	250µL of 2	250µL	500 pg/mL
# 4	250µL of 3	250µL	250 pg/mL
# 5	250µL of 4	250µL	125 pg/mL
# 6	250µL of 5	250µL	62.5 pg/mL



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

Streptavidin-HRP Conjugate - Pipette 11.94 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 60 µL of 200-fold concentrated stock solution to prepare working solution.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that standards 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 bag containing the desiccant pack, reseal.

3a. Add 30 µL of Sample Buffer (DB00) to every well that will be used.

3b. Add 100 µL of Dilution Buffer to Blank wells (A2, A3).

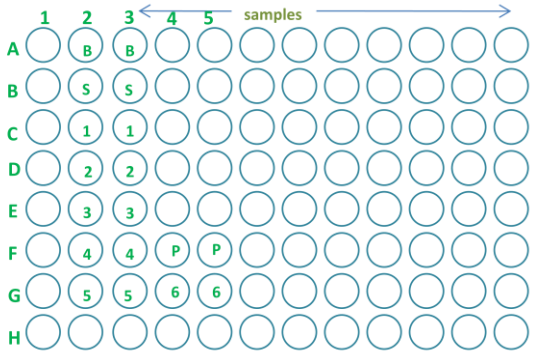
4. Add 100 µL of Standard (from B2, B3 to G2, G3 and G4, G5), sample, or positive control (F4, F5) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating 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 **Streptavidin-HRP Conjugate** working solution to each well. Incubate for 45 minutes on micro-plate shaker at room temperature.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 15-20 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 30 minutes, using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, 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 standards may result in inaccurate, low Mouse sRAGE levels. Such samples require further external predilution according to expected Mouse sRAGE values with Dilution Buffer in order to precisely quantify the actual Mouse sRAGE level.



CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant mouse RAGE/Fc Chimera.

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of SRAGE was 20 pg/mL (below lowest standard point).

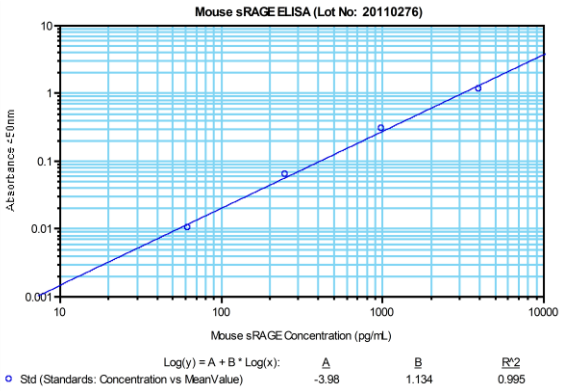
TYPICAL DATA

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

SRAGE STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)*
62.5	0.010
250	0.063
1000	0.294
4000	1.129

*Lot No.:

** Positive Control: 307 – 512 pg/mL



LINEARITY

To assess the linearity of the assay, pooled mouse research plasma samples were spiked with the recombinant protein and tested for recovery.

Sample + Recombinant sRAGE	Recovery (%)
Mouse plasma (25%) + Recombinant (75%)	63.8
Mouse plasma (50%) + Recombinant (50%)	53.4

SPECIFICITY

Proteins	Cross-reactivity
Mouse sRAGE	100%
Rat sRAGE	17%
Human sRAGE	0%

REFERENCES:

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