RAT/MOUSE SOLUBLE RECEPTOR FOR ADVANCED GLYCOSYLATION END PRODUCTS (sRAGE) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF sRAGE CONCENTRATIONS IN RAT OR MOUSE SERUM AND EDTA PLASMA

ALWAYS REFER TO LOT SPECIFIC PROTCOL 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:

ELISA NAME	RAT/MOUSE SOLUBLE RAGE ELISA
Catalog No.	SK00112-03
Lot NO.	
Formulation	96 T
Standard range	0.0256 – 400 ng/mL
Dynamic range	0.128 – 80 ng/mL
Sensitivity	0.05 ng/mL
Sample Volume	50 µL per well
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application.**
Sample Type	Serum, EDTA plasma
Specificity	Rat, Mouse
Calibration	Purified recombinant rat RAGE extracellular domain
Intra-assay Precision	6 - 8%
Inter-assay Precision	12 - 14%
Storage	2 – 8° C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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

DISCRIPTION

This Rat sRAGE ELISA kit contains the necessary components required for the quantitative measurement of recombinant and/or natural Rat sRAGE from serum and plasma in a competitive EIA format.

This immunoassay contains recombinant and biotinylated recombinant Rat sRAGE, and an antibody raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural sRAGE.

ASSAY OVERVIEW

Rat sRAGE ELISA employs the quantitatively competitive enzyme immunoassay technique in which rat sRAGE present in samples compete with a fixed amount of biotinylated rat sRAGE for sites on purified rabbit IgG specific against rat sRAGE. During the incubation, the rabbit IgG becomes bound to the goat anti-rabbit IgG pre-coated onto the microplate. Following a wash to remove any unbound antibody, standard, samples and biotin conjugate, a Streptavidin conjugated to horseradish-peroxidase (HRP) is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when stop solution is added. The intensity of the color measured is in inverse proportion to the amount of rat sRAGE bound in the initial step. The sample values are then read off the standard curve.

Rat sRAGE ELISA has been shown to accurately quantify the recombinant and natural rat sRAGE. Results obtained using natural rat sRAGE showed dose response curves that were parallel to the standard curves obtained using the kit standards.

PROCEDURAL LIMITATIONS

_ 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 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
R-Microplate - 96 well microplate pre-coated with polyclonal anti rabbit IgG Fc purified IgG.	**RM01**	**1 plate**
Rat sRAGE Standard – 400ng/vial of recombinant rat sRAGE in a buffered protein base with preservative; lyophilized.	**112-03-01**	**1 vial**
Biotin Solution Concentrate - 350µL/vial, 10-fold concentrate of rat sRAGE biotinylated with preservative; lyophilized.	**112-03-02**	**1 vial**
Antibody Concentrate – 350µL/vial, 10-fold concentrate of polyclonal purified IgG against rat sRAGE with preservative; lyophilized.	**112-03-03**	**1 vial**
Positive Control – one vial of recombinant rat sRAGE; lyophilized (optional).	**112-03-04**	**1 vial**
Streptavidin-HRP Conjugate - 120µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 60mL of buffered protein based solution with preservative. Ready to use.	**DB18**	**1 bottle**
HRP Diluent Solution - 12mL of buffered protein based solution with preservative.	**DB06**	**1 bottle**
Wash Buffer - 50mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11mL of TMB substrate solution.	**TMB01**	**1 bottle**

Stop Solution - 11mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 8 months. For longer storage, unopened Standard, Positive Control, Antibody Concentrate and Biotin Concentrate should be stored at -20 or -70°C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard, Biotin concentrated solution and Antibody concentrated solution SHOULD BE STORED at -20° C or -70° C for up to one month. Reconstituted Biotin Solution (350 µL) CAN NOT BE STORED at 2-8°C. Streptavidin-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 entire edge of zip-seal. Microplate may be stored for up to 6 months at 2-8°C.

ADDITIONAL MATERIALS 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.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

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

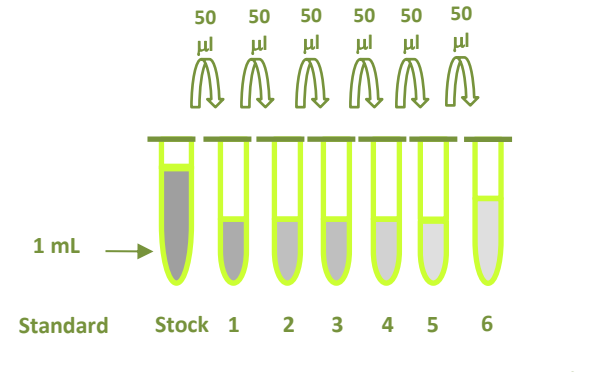
Rat or Mouse Serum and plasma samples may require a 2~4-fold dilution. A suggested 2-fold dilution is 60 µL sample + 60 µL Dilution Buffer. A suggested 4-fold dilution is 30 µL sample + 90 µL Dilution Buffer.
Optimal dilutions should be determined by each laboratory for each application with a sample pretest.
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.

Rat sRAGE Standard - Refer to vial label for reconstitution volume. Reconstitute the Rat **sRAGE** standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 400 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 200 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 **400 ng/mL** standard serves as the high standard.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.0ml	400 ng/ml
# 1	50µl of stock	200µl	80 ng/ml
# 2	50µl of 1	200µl	16 ng/ml
# 3	50µl of 2	200µl	3.2 ng/ml
# 4	50µl of 3	200µl	0.64 ng/ml
# 5	50µl of 4	200µl	0.128 ng/ml
# 6	50µl of 5	200µl	0.026 ng/ml



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.

Antibody Solution - Reconstitute the **Antibody Concentrate** with 350 µl of Dilution Buffer to produce a 10-fold concentrated stock solution. Transfer it to 3.15 mL of Dilution Buffer to prepare 1x Antibody Solution.

Biotin Solution - Reconstitute the **Biotin Solution Concentrate** with 350 µl of Dilution Buffer to make 10-fold concentrated solution. Transfer it to 3.15 mL of Dilution Buffer to prepare 1x Biotin Solution. **Note**: 1x working solution of Biotin Solution SHOULD BE STORED at -20° C or -70° C.

Streptavidin-HRP Conjugate - Transfer 120 µl of 100-fold concentrated stock solution to 11.88 mL of **HRP Diluent Solution (DB06)** to prepare working solution. **Note**: 1x working solution of Streptavidin-HRP Conjugate should be used within a few days **(protect from light)**.

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. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Leave two wells as Blank. ***DO NOT ADD ANY ANTIBODY OR BIOTIN SOLUTION INTO BLANK WELLS.***
4. Set two wells as total binding. Add 50 µl per well of **Dilution Buffer**.
5. Add 50 µl per well of **standard solution** to the appropriate wells. Add 50 µl per well of **Positive Control** into the appropriate wells. Add 50 µl per well of samples into appropriate wells.
6. Add 25µl per well of **1x Antibody Solution** into total binding, standard, positive control and sample wells. Cover with plate sealer and incubate on microplate shaker (250-300rpm) at room temperature for 2 hours. ***Note: DO NOT ASPIRATE AND WASH PLATE. PROCEED IMMEDIATELY TO THE NEXT STEP.***
7. Add 25 µl per well of **1x Biotin Solution** into total binding, standard, positive control and sample wells. Cover with plate sealer and incubate on microplate shaker (250-300rpm) at room temperature for 2 hours. ***Note: DO NOT ADD Biotin Solution to Blank wells.***
8. Aspirate wells and wash 4 times with 300 µl of **1x Wash Buffer**. Blot plate on absorbent paper to remove any residual buffer.
9. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate it on microplate shaker for 60 minutes at room temperature.
10. Aspirate and wash as step 8.
11. Add 100 µL of **Substrate Solution** to each well. Incubate for 1-10 minutes on microplate shaker at room temperature. **Protect from light**.
12. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. It is recommended to add the stop solution when the Total Binding or the lowest standard has developed a dark blue color.
13. Determine the optical density of each well within 15 minutes using a microplate reader set at 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average Blank optical density. It is recommended to use software capable of generating a four parameter

logistic (4-PL) curve-fit. The standard curve shows relationship between standard concentrations and corresponding O.D absorbance. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

SPECIFICITY

PROTEINS	CROSS-REACTIVITY (%)
Rat sRAGE	100
Mouse sRAGE	100
Human sRAGE	70
Human esRAGE C-terminal peptide	0

This assay recognizes both natural and recombinant rat sRAGE. The data also indicated that mouse serum samples were competitively bound to antibody that was used in this kit formulation condition. Its linear dilution curves were parallel to the standard curves obtained using the ELISA standard. This means mouse serum samples cross-react with Rat sRAGE ELISA kit.

TYPICAL DATA

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

WELL	OD450 READING	STANDARD (NG/ML)
Blank	0.076	
Total Binding	1.603	0
Standard 6	1.518	0.026
Standard 5	1.477	0.128
Standard 4	1.090	0.640
Standard 3	0.652	3.2
Standard 2	0.234	16
Standard 1	0.066	80
Standard S	0.012	400

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⬇
Add 50 µl of standard, samples, positive control to each well. Add 25 µL of 1x Antibody solution to each well used, except blanks. Incubate 2 hours on the plate shaker at RT. ***DO NOT ASPIRATE AND WASH PLATE. Proceed immediately to the next step.***
⬇
Add 25 µL of 1x Biotin Solution to each well used, except blanks. Incubate 2 hours on the plate shaker at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Streptavidin-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 4-8 min on the plate shaker at RT. **Protect from light**.
⬇
Add 100 µl Stop Solution to each well. Read 450nm within 15 minutes.

REFERENCES

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