BioVendor Group

ENG

Product Data Sheet:

HUMAN SRAGE ELISA

Catalogue number:
RD191116200R

For research use only!

BioVendor R&D

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CONTENTS

1. INTENDED USE

The RD191116200R Human sRAGE ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human sRAGE (soluble receptor for advanced glycation end products).

Features

- **It is intended for research use only**
- The total assay time is less than 4.5 hours
- The kit measures sRAGE in serum and plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- Standard is recombinant protein based
- Quality Controls are human serum based
- Components of the kit are provided ready to use, concentrated or lyophilized

2. STORAGE, EXPIRATION

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 9.

3. INTRODUCTION

The receptor for advanced glycation end products (RAGE) belongs to the immunoglobulin superfamily and consists of the 319 amino acids-long extracellular region, hydrophobic transmembrane domain and 43-amino acid, highly charged, cytoplasmic tail. The extracellular region of the receptor is composed from three parts: one Ig-like V-type domain and two Ig-like C-type domains. It was shown that the aminoterminal part of the molecule is responsible for binding of the ligands. On the other hand, the carboxyterminal part is responsible for mediating of the signalling actions.

RAGE expression decreases throughout development in several types of cells, including neurons, endothelium, pericytes, smooth muscle cells, mononuclear phagocytes, cardiac myocytes, hepatocytes, renal glomerular epithelial cells or podocytes and Muller and bipolar ganglion cells of the retina. Interestingly, the expression of the RAGE remains at high levels in the lung alveolar epithelial cells in adult tissues.

RAGE is connected to several diseases and pathological states (e.g., diabetic complication, vascular and inflammatory diseases). It was characterized as receptor for advanced glycation end products (AGEs), but this receptor can also bind other ligands such as proinflammatory S100 proteins/calgranulins, amphoterin or amyloid beta-peptide. AGEs are groups of the substances formed via nonenzymatic reaction of reducing sugars with free amino groups of proteins, lipids and nucleic acids. AGEs are formed in the body constantly throughout life and accumulate over time. It is natural that in diabetes, when glucose is more available, the formation of AGEs is increased. Their effect in the body can be divided into (1) the crosslinking of the extracellular matrix proteins and thus altering the cellular structure and (2) interaction of AGEs with their receptor on the cell surfaces and thus altering cellular function via signalling. The binding of various proinflammatory molecules to the cell-surface RAGE triggers an intracellular signalling pathway. The target is nuclear factor-κB (NF- κB) which promotes enhanced transcription of proinflammatory genes and also up-regulates the transcription of RAGE.

In addition to full-length RAGE, several truncated isoforms of this receptor have been found. First, there is a splice variant encoding molecule that is lacking N-terminal region and, second, is the variant missing the C-terminus. The RAGE splice variant lacking C-terminal part is missing the transmembrane region, and after secretion to the extracellular environment, it circulates in blood as endogenous secretory RAGE (esRAGE). esRAGE successfully binds to AGEs and, due to this ability, it can act as an antagonist by preventing the activation of cell-surface full-length RAGE. In sera, esRAGE as well as another soluble version of RAGE possibly created by proteolytical cleavage by matrix metalloproteases can be detected. This potential mechanism was initially described in mouse, but it could be present in humans, too. Because of the possible neutralization effect of soluble RAGE, studies have examined the significance of sRAGE serum concentration in patients with various pathological conditions. Decreased level of sRAGE is a biomarker for deficient and/or altered inflammatory control in humans. It was shown that reduced level of sRAGE is associated with higher risk of coronary disease. In Alzheimer disease there is a decrease in serum sRAGE in comparison with patients with vascular dementia and controls. In essential hypertensia, it has been shown that sRAGE concentration in serum was inversely associated with pulse pressure. On the other hand, an increased level of serum sRAGE was found in patients with end-stage renal disease and acute lung injury.

<u>Areas of investigation:</u>

Metabolic syndrome

Glucose and lipid homeostase

Renal diseases

4. TEST PRINCIPLE

In the Biovendor Human sRAGE ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human sRAGE antibody. After 120 minutes incubation and washing, biotin labelled polyclonal anti-human sRAGE antibody is added and incubated with captured sRAGE for 60 minutes. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of sRAGE. A standard curve is constructed by plotting absorbance values versus sRAGE concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

5. PRECAUTIONS

- **For professional use only**
- Wear gloves and laboratory coats when handling immunodiagnostic materials
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents
- This kit contains components of animal origin. These materials should be handled as potentially infectious
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary
- The materials must not be pipetted by mouth

6. TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware
- Use deionized (distilled) water, stored in clean containers
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

7. REAGENT SUPPLIED

Kit Components	State	Quantity
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody Conc. (100x)	concentrated	0.13 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	1 vial
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
Dilution Buffer	ready to use	20 ml
Biotin-Ab Diluent	ready to use	13 ml
Wash Solution Conc. (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision pipettes to deliver 10-1000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
- Vortex mixer
- Orbital microplate shaker capable of approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
- Software package facilitating data generation and analysis (optional)

9. PREPARATION OF REAGENTS

All reagents need to be brought to room temperature prior to use.

Always prepare only the appropriate quantity of reagents for your test.

Do not use components after the expiration date marked on their label.

Assay reagents supplied ready to use:

Antibody Coated Microtiter Strips

Stability and storage:

Return the unused strips to the provided aluminium zip-sealed bag with desicant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8°C and protected from the moisture.

Dilution Buffer

Biotin-Ab Diluent

Streptavidin-HRP Conjugate

Substrate Solution

Stop Solution

Stability and storage:

Opened reagents are stable 3 months when stored at 2-8°C.

Assay reagents supplied concentrated or lyophilized:

Human sRAGE Master Standard

Refer to Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!

Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of the human sRAGE in the stock solution is **3 200 pg/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	-	3 200 pg/ml
250 µl of stock	250 µl	1 600 pg/ml
250 µl of 1 600 pg/ml	250 µl	800 pg/ml
250 µl of 800 pg/ml	250 µl	400 pg/ml
250 µl of 400 pg/ml	250 µl	200 pg/ml
250 µl of 200 pg/ml	250 µl	100 pg/ml
250 µl of 100 pg/ml	250 µl	50 pg/ml

Prepared Standards are ready to use, do not dilute them.

Stability and storage:

The reconstituted Standard stock solution must be used immediately or aliquoted and frozen at - 20°C for 3 months. Avoid repeated freezing/thawing cycles.

Do not store the diluted Standard solutions.

Quality Controls HIGH, LOW

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Control concentration!!!

Reconstitute each Quality Control (HIGH and LOW) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).

Reconstituted Quality Controls are ready to use, do not dilute them.

Stability and storage:

Do not store the reconstituted Quality Controls.

Note:

Concentration of analyte in Quality Controls need not be anyhow associated with normal and/or pathological concentrations in serum or another body fluid. Quality Controls serve just for control that the kit works in accordance with PDS and CoA and that ELISA test was carried out properly.

Biotin Labelled Antibody Conc. (100x)

Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (100x) with 99 parts Biotin-Ab Diluent. Example: 10 µl of Biotin Labelled Antibody Concentrate (100x) + 990 µl of Biotin-Ab Diluent for 1 strip (8 wells).

Stability and storage:

Opened Biotin Labelled Antibody Concentrate (100x) is stable 3 months when stored at 2-8°C.

Do not store the diluted Biotin Labelled Antibody solution.

Wash Solution Conc. (10x)

Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 100 ml of Wash Solution Concentrate (10x) + 900 ml of distilled water for use of all 96-wells.

Stability and storage:

The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

10. PREPARATION OF SAMPLES

The kit measures human sRAGE in serum and plasma (EDTA, citrate, heparin).

Samples should be assayed immediately after collection or should be stored at -20°C. Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Dilute samples 3x with Dilution Buffer just prior to the assay, e.g. 50 µl of sample + 100 µl of Dilution Buffer for singlets, or preferably 100 µl of sample + 200 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:

Serum and plasma samples should be stored at -20°C, or preferably at -70°C for long-term storage.

Do not store the diluted samples.

See Chapter 13 for stability of serum and plasma samples when stored at 2-8°C, effect of freezing/thawing and effect of sample matrix (serum/plasma) on the concentration of sRAGE.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.

11. ASSAY PROCEDURE

1. Pipet **100 µl** of Standards, Quality Controls, Dilution Buffer (=Blank) and diluted samples, preferably in duplicates, into the appropriate wells. See *Figure 1* for example of work sheet.
2. Incubate the plate at room temperature (ca. 25°C) for **2 hours**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add **100 µl** of Biotin Labelled Antibody solution into each well.
5. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add **100 µl** of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for **30 minutes,** shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
10. Add **100 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
11. Incubate the plate for **10 minutes** at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C. Do not shake the plate during the incubation.
12. Stop the colour development by adding **100 µl** of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 - 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm.
    The absorbance should be read within 5 minutes following step 12.

Note: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine sRAGE concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were “in range” at 450 nm.

Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat four times. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	**Standard 3 200**	**QC HIGH**	Sample 7	Sample 15	Sample 23	Sample 31
B	**Standard 1 600**	**QC LOW**	Sample 8	Sample 16	Sample 24	Sample 32
C	**Standard 800**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
D	**Standard 400**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
E	**Standard 200**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
F	**Standard 100**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
G	**Standard 50**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
H	**Blank**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38

Figure 1: Example of a work sheet.

12. CALCULATIONS

Most microtiter plate readers perform automatic calculations of analyte concentration. The Standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of sRAGE (pg/ml) in samples.

Alternatively, the *logit log* function can be used to linearize the standard curve, i.e. *logit* of the mean absorbance (Y) is plotted against log of the known concentration (X) of Standards.

The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay, e.g. 300 pg/ml (from standard curve) x 3 (dilution factor) = 900 pg/ml.

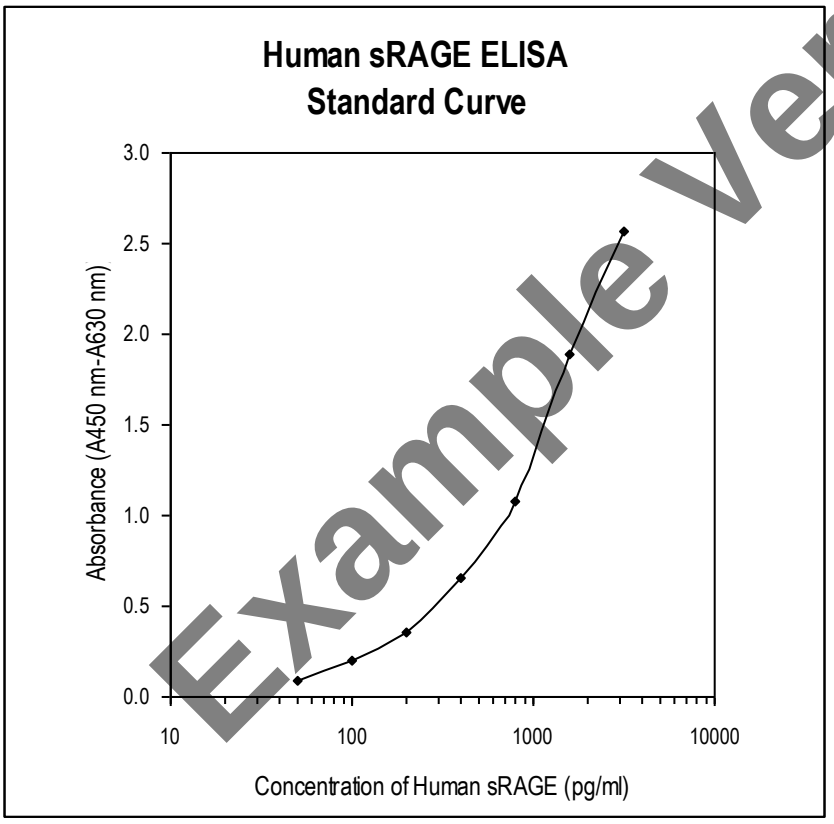

Figure 2: Typical Standard Curve for Human sRAGE ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human sRAGE ELISA are presented in this chapter.

Sensitivity

Limit of detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{blank} + 3xSD_{blank}$) is calculated from the real human sRAGE values in wells and is: 19.2 pg/ml.

* Dilution Buffer is pipetted into Blank wells.

Limit of assay

Results exceeding human sRAGE level of 3 200 pg/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the sRAGE concentration.

Specificity

The antibodies used in this ELISA are specific for human sRAGE. Determination of human sRAGE does not interfere with hemoglobin (3 %), bilirubin (170 µmol/l) and triglycerides (5.0 mmol/l).

Sera of several mammalian species were measured in the assay. See results below.

For details please contact us at info@biovendor.com.

Mammalian serum sample	Observed crossreactivity
Bovine	no
Cat	no
Dog	no
Goat	no
Hamster	no
Horse	no
Monkey	no
Mouse	no
Pig	no
Rabbit	no
Rat	no
Sheep	no

Presented results are multiplied by respective dilution factor.

Precision

Intra-assay (Within-Run) (n=8)

Sample	Mean (pg/ml)	SD (pg/ml)	CV (%)
1	1 595.7	41.7	2.6
2	1 075.5	57.1	5.3

Inter-assay (Run-to-Run) (n=6)

Sample	Mean (pg/ml)	SD (pg/ml)	CV (%)
1	1 644.6	90.0	5.5
2	4 742.7	419.1	8.8

Spiking Recovery

Serum samples were spiked with different amounts of human sRAGE and assayed.

Sample	Observed (pg/ml)	Expected (pg/ml)	Recovery O/E (%)
1	1 831.9	-	-
	6 228.6	6 631.9	93.9
	3 633.3	4 231.9	85.9
	2 815.9	3 031.9	92.9
2	2 855.0	-	-
	7 498.2	7 655.0	98.0
	4 668.5	5 255.0	88.8
	4 218.7	4 055.0	104.0

Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (pg/ml)	Expected (pg/ml)	Recovery O/E (%)
1	-	4571.1	-	-
	2x	2 368.4	2 285.6	103.6
	4x	1 287.2	1 142.8	112.6
	8x	569.0	571.4	99.6
2	-	9 068.4	-	-
	2x	4 166.6	4 534.2	91.9
	4x	2 228.6	2 267.1	98.3
	8x	1 170.3	1 133.6	103.2

Effect of sample matrix

EDTA, citrate and heparin plasmas were compared to respective serum samples from the same 10 individuals. Results are shown below:

Volunteer No.	Serum (pg/ml)	Plasma (pg/ml)		
		EDTA	Citrate	Heparin
1	818.2	955.5	667.2	783.1
2	710.8	888.0	712.9	715.0
3	1 283.9	1 055.5	988.2	1 171.7
4	2 075.0	2 023.3	2 137.0	2 032.7
5	1 277.8	1 167.6	1 047.3	1 133.0
6	1 974.1	1 901.9	1 813.3	1 836.6
7	1 563.3	1 186.0	1 135.0	1 190.0
8	2 126.2	1 780.5	2 149.9	2 025.7
9	1 752.6	1 249.2	1 386.3	1 417.2
10	1 677.42	1 567.5	1 462.3	1 546.9
Mean (pg/ml)	**1 525.9**	**1 377.5**	**1 349.9**	**1 385.2**
Mean Plasma/Serum (%)	**-**	**90.3**	**88.5**	**90.8**
Coefficient of determination R^2	**-**	**0.81**	**0.91**	**0.94**

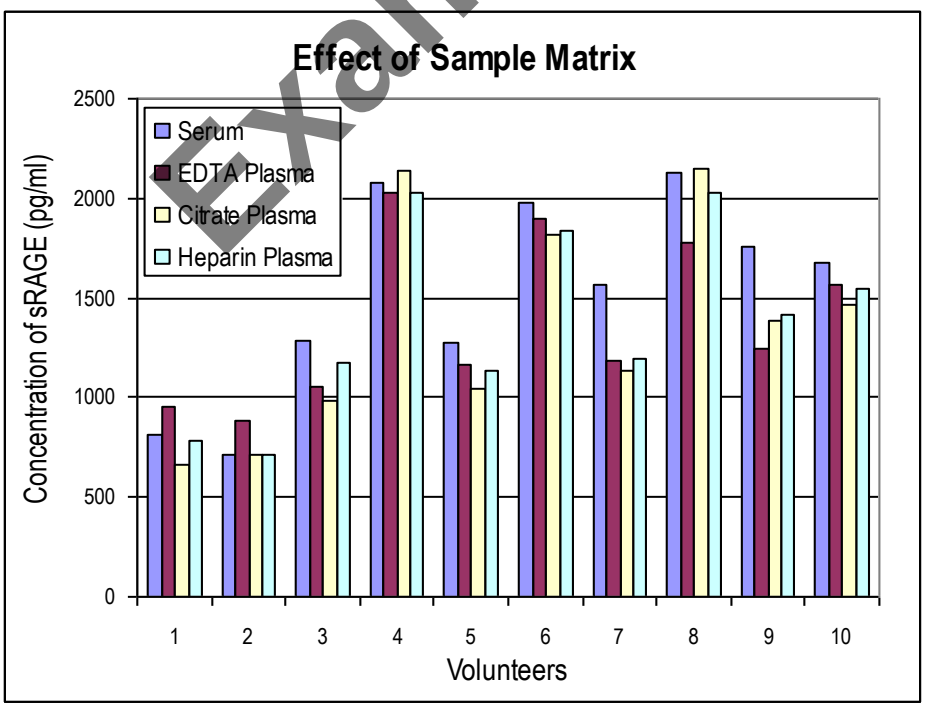

Figure 3: sRAGE levels measured using Human sRAGE ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

Stability of samples stored at 2-8°C

Samples should be stored at -20°C. However, no significant decline in concentration of human sRAGE was observed in serum and plasma samples after 7 days when stored at 2-8°C. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and sodium azide, resulting in the final concentration of 0.03% and 0.1%, respectively.

Sample	Incubation Temp, Period	Serum (pg/ml)	Plasma (pg/ml)		
			EDTA	Citrate	Heparin
1	-20°C	1 108.3	959.0	1 248.5	905.6
	2-8°C, 1 day	899.9	841.8	923.8	1 007.7
	2-8°C, 7 days	1 041.5	902.0	1 064.5	1 018.9
2	-20°C	1 222.2	1 260.7	1 233.2	1 262.9
	2-8°C, 1 day	1 315.9	1 305.9	1 184.9	1 340.9
	2-8°C, 7 days	1 564.0	1 274.2	1 214.2	1 139.7
3	-20°C	1 754.3	1 659.9	1 807.8	1 482.2
	2-8°C, 1 day	1 632.4	1 564.7	1 707.9	1 582.3
	2-8°C, 7 days	1 627.9	1 569.5	1 920.1	1 680.7

Effect of Freezing/Thawing

No significant decline was observed in concentration of human sRAGE in serum samples after repeated (5x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

Sample	Number of f/t cycles	Serum (pg/ml)	Plasma (pg/ml)		
			EDTA	Citrate	Heparin
1	1x	831.1	703.3	920.3	926.5
	3x	782.1	881.6	578.9	938.1
	5x	984.6	839.2	898.2	1 007.1
2	1x	623.2	585.6	614.9	586.9
	3x	744.4	737.6	594.2	851.8
	5x	866.0	704.1	584.5	494.3
3	1x	1 435.4	1 537.8	1 536.5	1 292.8
	3x	1 414.0	1 479.9	1 251.4	1 389.9
	5x	1 430.6	1 335.9	1 586.4	1 576.6

14. DEFINITION OF THE STANDARD

The recombinant human sRAGE is used as the Standard. The human sRAGE, produced in *E.coli*, is 36.5 kDa protein containing methionyl 339 amino acid residues of the human RAGE.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 191 unselected donors (95 men + 96 women) 3-88 years old were assayed with the Biovendor Human sRAGE ELISA in our laboratory.

Age and sex dependent distribution of sRAGE

Sex	Age years	n	Mean	SD	Min.	Max.	Median
			sRAGE (pg/ml)				
Men	3 - 19	8	798.6	138.8	541.5	970.8	832.1
	21 - 49	30	717.7	260.7	349.5	1 738.3	692.0
	50- 88	57	714.1	232.6	357.5	1 286.3	696.4
Women	3– 18	9	813.8	215.3	527.8	1 281.0	826.0
	20 - 49	39	900.2	365.9	435.9	2 517.0	827.5
	50 - 84	48	835.5	288.4	419.1	2 131.6	769.8

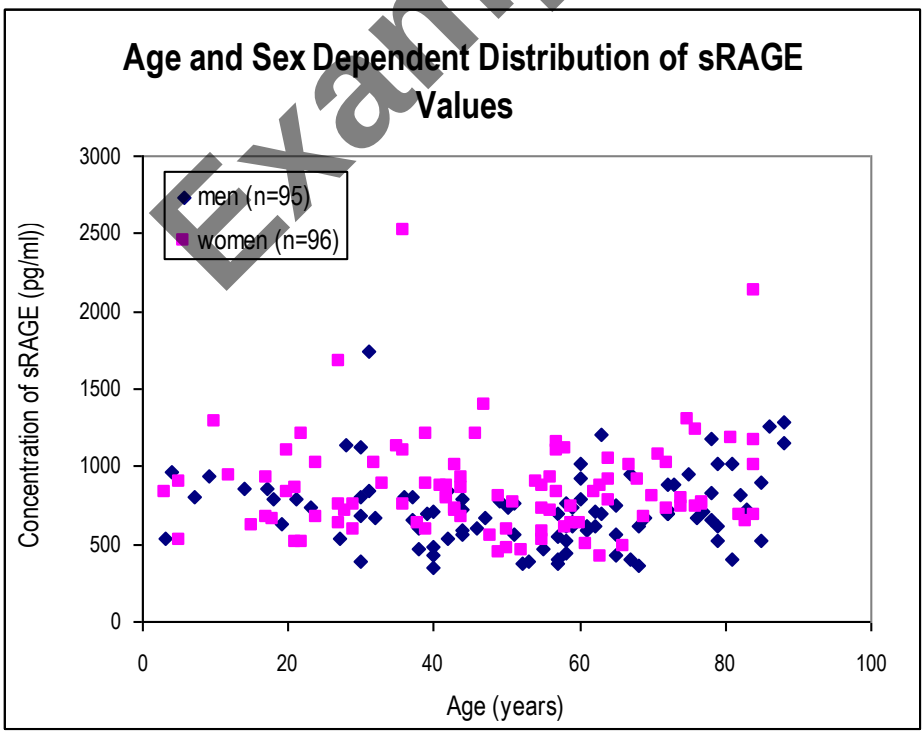

Figure 4: Human sRAGE concentration plotted against donor age and sex.

Reference range

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and pathological reference ranges for sRAGE levels with the assay.

16. METHOD COMPARISON

The Biovendor Human sRAGE ELISA was compared to another commercial ELISA immunoassay, by measuring 27 serum samples. The following correlation graph was obtained.

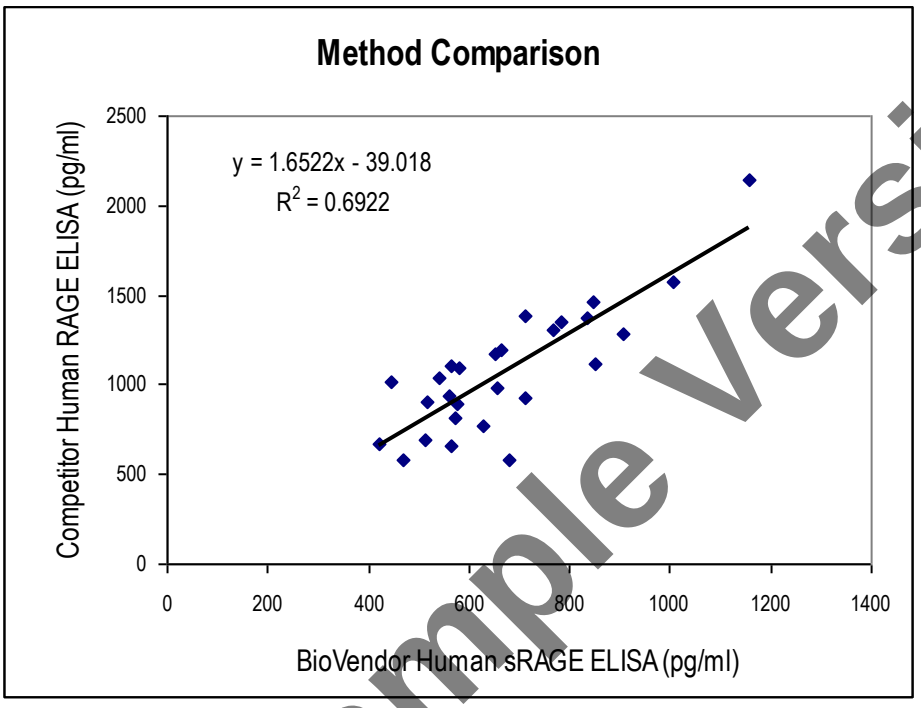

Figure 5: Method comparison.

17. TROUBLESHOOTING AND FAQS

Weak signal in all wells

Possible explanations:

- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper wavelength when reading absorbance

High signal and background in all wells

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30°C

High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing
- Improper mixing Standards, Quality Controls or samples

18. REFERENCES

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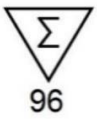
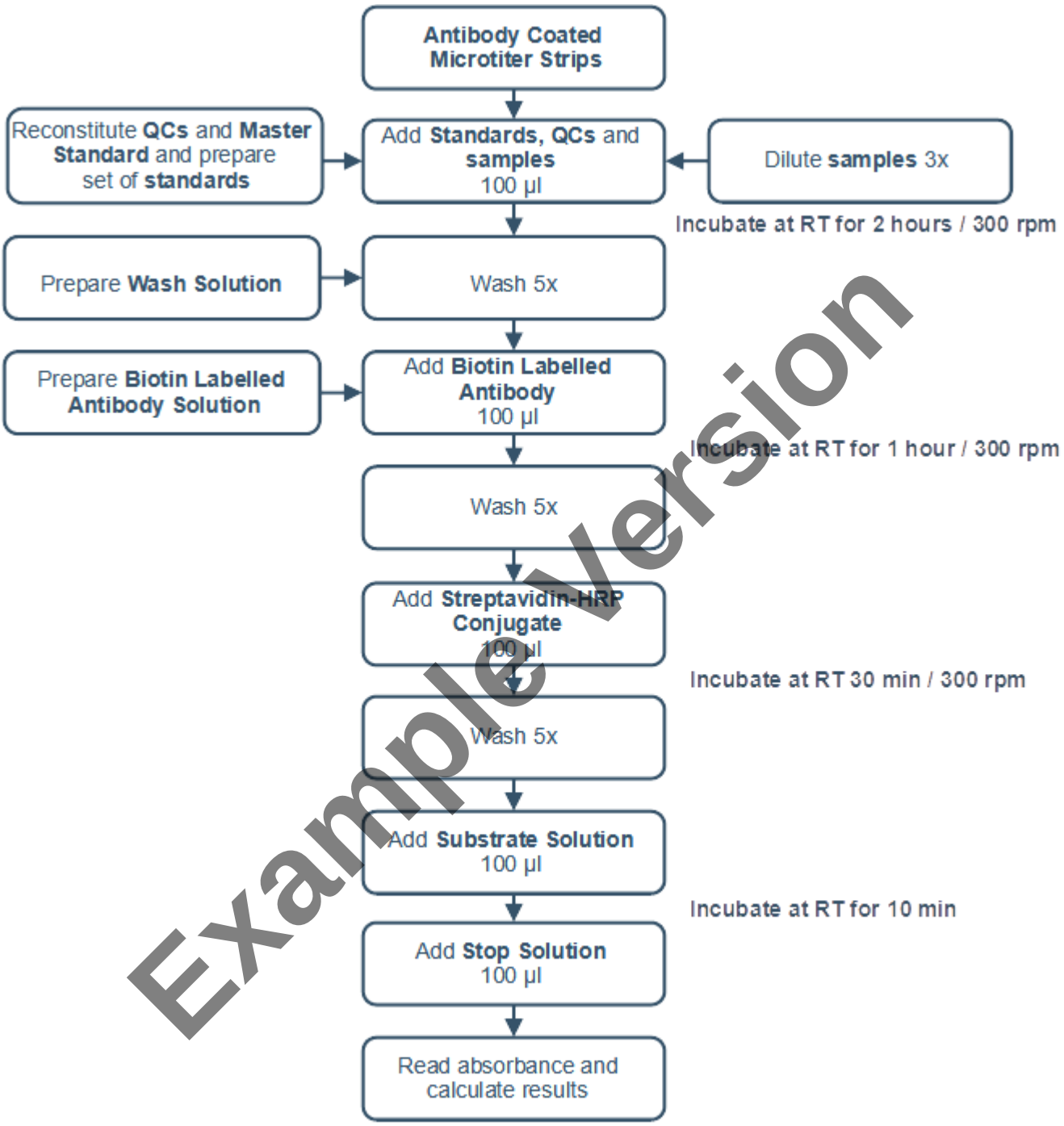
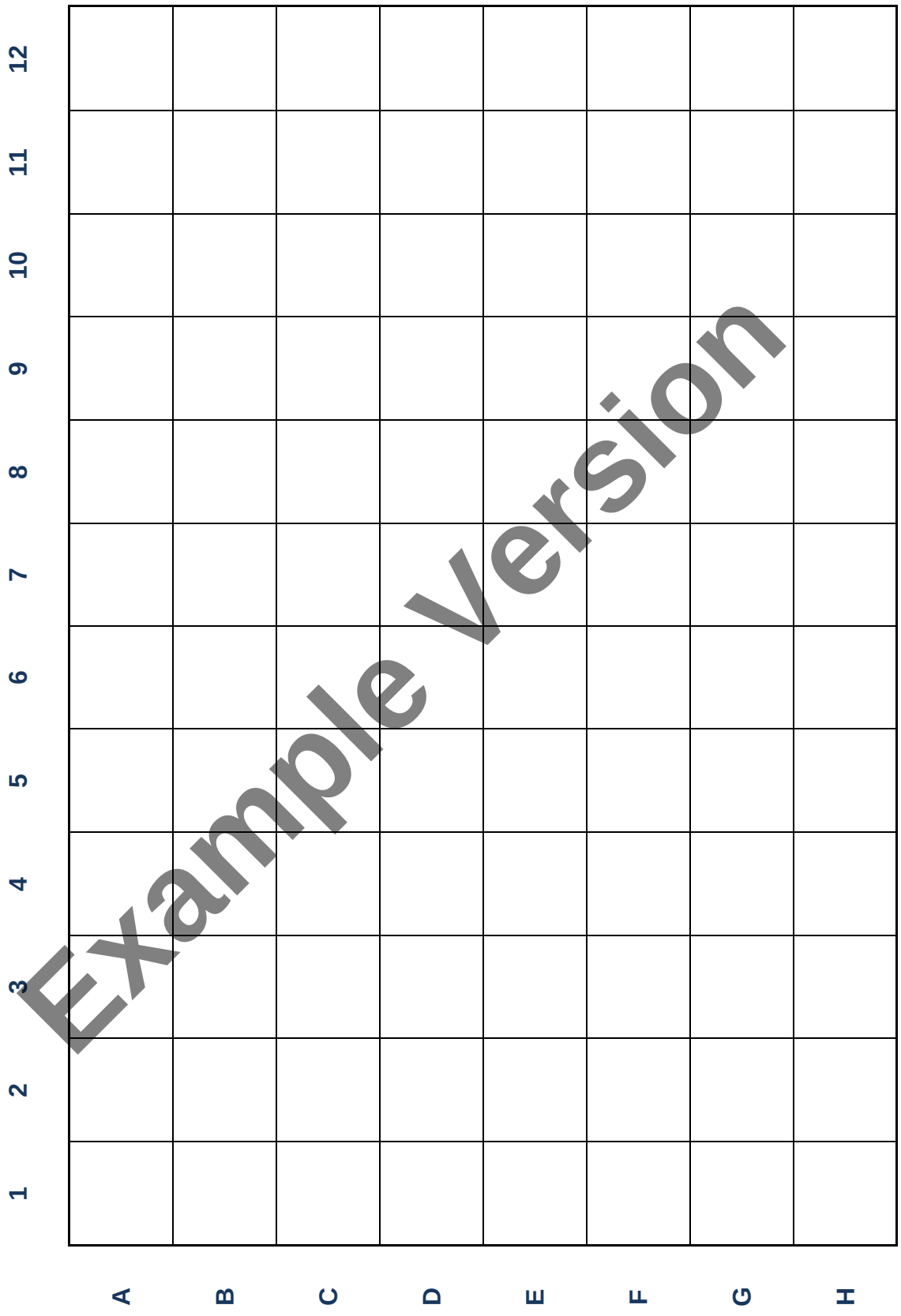
For more references on this product see our web pages at www.biovendor.com.

19. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks

20. ASSAY PROCEDURE - SUMMARY





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