

ENG

Instructions for Use:
HUMAN S100A12 ELISA

Catalogue number:
RD191221200R

For research use only!

 **BioVendor**
R&D[®]



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HISTORY OF CHANGES

Previous version	Current version
ENG.009.A	ENG.010.A
Symbol indicating the manufacturer added.	

1. INTENDED USE

The RD191221200R Human S100A12 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human S100A12.

Features

- **It is intended for research use only**
- The total assay time is less than 3.5 hours
- The kit measures S100A12 in serum, BALF and stool samples
- Extraction Buffer (Cat. No.: C005821) needed for extraction of stool samples is not included and can be obtained from BioVendor. For details please contact us at info@biovendor.com
- Assay format is 96 wells
- Quality Controls are human serum based
- Standard is recombinant protein 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

S100A12 (EN-RAGE, calgranulin) is a member of the S100 protein family, which, in humans, consists of twenty five EF-hand (alpha helix-loop-alpha helix), calcium-binding proteins, of which the vast majority is in homodimer, heterodimer or more complex forms [10]. Mature S100A12 consists of 91 amino acids (Mw 10.4 kDa); it is predominantly expressed and secreted by neutrophil granulocytes and to a lower extent, it was found in monocytes.

Hofmann et al. (1999) reported that RAGE is a central cell surface receptor for S100A12, which they referred to as EN-RAGE (Extracellular Newly identified RAGE-binding protein), and related members of the S100/calgranulin superfamily [20]. Interaction of EN-RAGE (S100A12) with cellular RAGE on endothelium, mononuclear phagocytes, and lymphocytes triggered cellular activation, with generation of key proinflammatory mediators [12]. In murine models, blockade of EN-RAGE/RAGE quenched delayed-type hypersensitivity and inflammatory colitis by arresting activation of central signaling pathways and expression of inflammatory gene mediators.

With regard to its inflammatory properties, S100A12 was already described as a promising marker for many diseases in humans such as neurodegenerative diseases, atherosclerosis, cancerogenesis, osteoarthritis, familial mediterranean fever and idiopathic pulmonary fibrosis. Plasma S100A12 level is associated with cardiovascular disease in hemodialysis patients. S100A12 and its receptor RAGE are found at high concentrations in pulmonary tissue and bronchoalveolar lavage fluid in acute lung injury. S100A12 expression reflects neutrophil activation during lung inflammation [18]. Faecal S100A12 is a novel non-invasive marker distinguishing inflammatory bowel disease (IBD) from irritable bowel syndrome (IBS). Furthermore, S100A12 reflects inflammatory activity of chronic IBD. As a marker of neutrophil activation, faecal S100A12 may significantly improve the arsenal of non-invasive biomarkers of intestinal inflammation [17].

Areas of investigation:

Immune response, infection and inflammation
Renal disease
Lipid metabolism
Neurodegenerative disorders
Oncology

4. TEST PRINCIPLE

In the BioVendor Human S100A12 ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human S100A12 antibody. After 60 min incubation and washing, biotin-labelled polyclonal anti-human S100A12 antibody is added and incubated with captured S100A12 for 60 min. After another washing, streptavidin-HRP conjugate is added. After 30 min 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 S100A12. A standard curve is constructed by plotting absorbance values against 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
- 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	2 vials
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
Biotin-Ab Diluent	ready to use	13 ml
Dilution Buffer	ready to use	50 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 5-1000 μl with disposable tips
- Multichannel pipette to deliver 100 μl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtiter 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.

Centrifuge liquid containing microtube vials before opening.

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 desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8 °C and protected from the moisture.

Streptavidin-HRP Conjugate

Biotin-Ab Diluent

Dilution Buffer

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 S100A12 Master Standard

Refer to the 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 S100A12 in the stock solution is 4 ng/ml.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	-	4 ng/ml
250 µl of stock	250 µl	2 ng/ml
250 µl of 2 ng/ml	250 µl	1 ng/ml
250 µl of 1 ng/ml	250 µl	0.5 ng/ml
250 µl of 0.5 ng/ml	250 µl	0.25 ng/ml
250 µl of 0.25 ng/ml	250 µl	0.125 ng/ml
250 µl of 0.125 ng/ml	250 µl	0.063 ng/ml

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

Stability and storage:

Do not store the reconstituted Master Standard and/or 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).

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

Stability and storage

Do not store 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 kit works in accordance with Instructions for use 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) to 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 S100A12 in serum, BALF and stool samples. The kit is not validated for plasma samples.

Samples should be assayed immediately after collection or should be stored frozen. Thoroughly mix thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic serum samples.

Serum samples

Dilute samples 100x with Dilution Buffer just prior to the assay (e.g. 5 µl of sample + 495 µl of Dilution Buffer). **Mix well** (not to foam). Vortex is recommended.

Stability and storage:

Samples should be stored at -20°C, or preferably at -70°C for long-term storage. Avoid repeated freeze/thaw cycles. **Do not store the diluted samples.**

See Chapter 13 for stability of serum samples when stored at 2-8°C and effect of freezing/thawing on the concentration of S100A12.

BALF samples

Dilute samples 5x with Dilution Buffer just prior to the assay (e.g. 30 µl of sample + 120 µl of Dilution Buffer for singlets, or preferably 50 µl of sample + 200 µl of Dilution Buffer for duplicates). **Mix well** (not to foam). Vortex is recommended.

Stability and storage:

Samples should be stored at -20°C, or preferably at -70°C for long-term storage. Avoid repeated freeze/thaw cycles. **Do not store the diluted samples.**

Stool samples

Collection and extraction:

Collect 50 to 100 mg of stool for extraction procedure – add BioVendor Extraction Buffer (Cat. No.: C005821) to polypropylene tube with known weight of a stool samples giving dilution factor 50x, e.g. if stool weight is 55 mg add 2695 μ l of Extraction Buffer [55 (weight) x 50 (dilution factor) – 55 (weight) = 2695 μ l]. Homogenize the samples on a vortex at high speed for 30 minutes and centrifuge for 5 minutes at 3000 g. Use supernatant for analysis in ELISA.

Dilute stool extracts 20x with Dilution Buffer just prior to the assay, e.g. 10 μ l of sample + 190 μ l of Dilution Buffer for singlets, or preferably 15 μ l of sample + 285 μ l of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:

Stool samples should be stored at 2-8°C up to 6 days and for long-term storage should be stored at -20°C, or preferably at -70°C. Extract should be stored at -20°C, or preferably at -70°C for at least 3 months. Avoid repeated freeze/ thaw cycles

Do not store the diluted samples.

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, reconstituted 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 **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 3-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 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 3-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 3-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. (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 1: If some samples and standard/s have absorbance 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 S100A12 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 twice. 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 4	QC HIGH	Sample 7	Sample 15	Sample 23	Sample 31
B	Standard 2	QC LOW	Sample 8	Sample 16	Sample 24	Sample 32
C	Standard 1	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
D	Standard 0.5	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
E	Standard 0.25	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
F	Standard 0.125	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
G	Standard 0.063	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 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 S100A12 (ng/ml) in samples.

Alternatively, the *logit log* function can be used to linearize the standard curve, i.e. *logit* of 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. 0.25 ng/ml (from standard curve) x 100 (dilution factor) = 25 ng/ml.

For stool samples concentration must be multiplied by extraction dilution factor and respective ELISA dilution factor before assaying, e.g. 0.25 ng/ml (from standard curve) x 20 (ELISA dilution factor) x 50 (extraction dilution factor) = 250 ng/ml

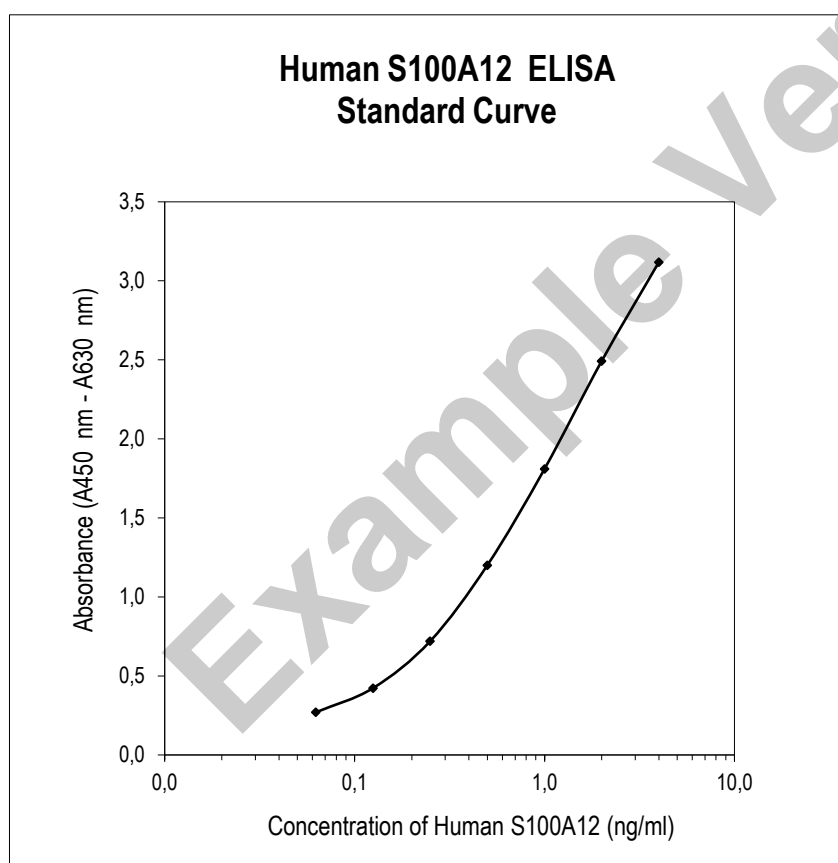


Figure 2: Typical Standard Curve for Human S100A12 ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human S100A12 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: $A_{\text{blank}} + 3xSD_{\text{blank}}$) is calculated from the real S100A12 values in wells and is 0.02 ng/ml.

*Dilution Buffer is pipetted into blank wells.

Limit of assay

Samples with absorbances exceeding the absorbance of the highest standard should be measured again with higher dilution. The final concentration of samples calculated from the standard curve must be multiplied by the respective dilution factor.

Specificity

The antibodies used in this ELISA are specific for human S100A12 with no detectable crossreactivities to other human recombinant S100 proteins such as S100A1, A2, A3, A4, A5, A6, A7, A10, A11, A12, A13, A14, A15, A16, S100-B and S100-G protein at 200 ng/ml.

Determination of S100A12 exhibits no interference by haemoglobin (1.0 mg/ml), bilirubin (170 $\mu\text{mol/l}$) 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	yes
Rabbit	no
Rat	no
Sheep	no

Presented results are multiplied by respective dilution factor

Precision

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

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	27.04	0.88	3.3
2	58.09	2.18	3.8

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

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	13.07	0.46	3.6
2	56.01	3.22	5.8

Spiking Recovery

Samples were spiked with different amounts of human S100A12 and assayed.

Sample	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
serum 1	14.90	-	-
	22.05	21.15	104.1
	28.25	27.40	103.1
	39.85	39.90	99.9
serum 2	30.25	-	-
	46.25	42.75	108.2
	59.20	55.25	107.1
	89.75	80.25	111.8
stool 1	92.5	-	-
	155.5	155.0	100.3
	220.0	217.5	101.1
	311.5	342.5	90.9
stool 2	81.0	-	-
	110.0	112.5	97.8
	136.5	143.5	95.1
	188.0	206.0	91.3
BALF	1.09	-	-
	1.72	1.72	100.0
	2.31	2.34	98.7
	3.60	3.59	100.3

Linearity

Samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
serum 1	-	44.40	-	-
	2x	22.20	22.20	100.0
	4x	11.40	11.10	102.7
	8x	5.70	5.55	102.7
serum 2	-	65.15	-	-
	2x	31.30	32.58	96.1
	4x	15.30	16.29	93.9
	8x	7.90	8.14	97.0
stool 1	-	251.0	-	-
	2x	112.0	125.5	89.4
	4x	54.5	62.8	86.9
	8x	28.0	31.4	89.2
stool 2	-	975.0	-	-
	2x	460.0	487.5	94.4
	4x	241.0	243.8	98.9
	8x	127.0	121.9	104.2
BALF	-	8.54	-	-
	2x	4.02	4.27	94.3
	4x	2.02	2.14	94.8
	8x	0.93	1.07	87.1

Stability of samples stored at 2-8°C

Samples should be stored at -20 °C. However, no decline in concentration of S100A12 was observed in serum samples after 7 days when stored at 2-8 °C. To avoid microbial contamination, samples were treated with ϵ -aminocaproic acid and thimerosal, resulting in the final concentration of 0.03% and 0.05%, respectively.

Sample	IncubationTemp, Period	Serum (ng/ml)
1	-20°C	55.73
	2-8°C, 1 day	57.73
	2-8°C, 7 days	62.48
2	-20°C	51.97
	2-8°C, 1 day	47.79
	2-8°C, 7 days	49.02
3	-20°C	30.64
	2-8°C, 1 day	29.60
	2-8°C, 7 days	32.00

Effect of Freezing/Thawing

No decline was observed in concentration of human S100A12 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 (ng/ml)
1	1x	15.64
	3x	13.16
	5x	15.21
2	1x	7.27
	3x	7.83
	5x	7.47
3	1x	16.73
	3x	21.45
	5x	17.84

14. DEFINITION OF THE STANDARD

As a standard in this assay, recombinant human S100A12 is used. The protein has 101 amino acids and molecular weight of 11.63 kDa.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum from 150 unselected donors (83 women + 67 men), 20-65 years old were assayed with BioVendor Human S100A12 ELISA kit in our laboratory.

Age and sex dependent distribution of S100A12

Sex	Age (years)	n	Mean	Median	SD	Min.	Max.
			S100A12 (ng/ml)				
Men	20-50	73	35.51	28.06	27.15	3.38	170.72
	51-65	10	42.99	37.79	12.68	21.71	66.56
Women	22-49	59	33.77	30.75	18.25	7.14	91.22
	52-61	8	17.58	14.55	8.51	6.42	31.94

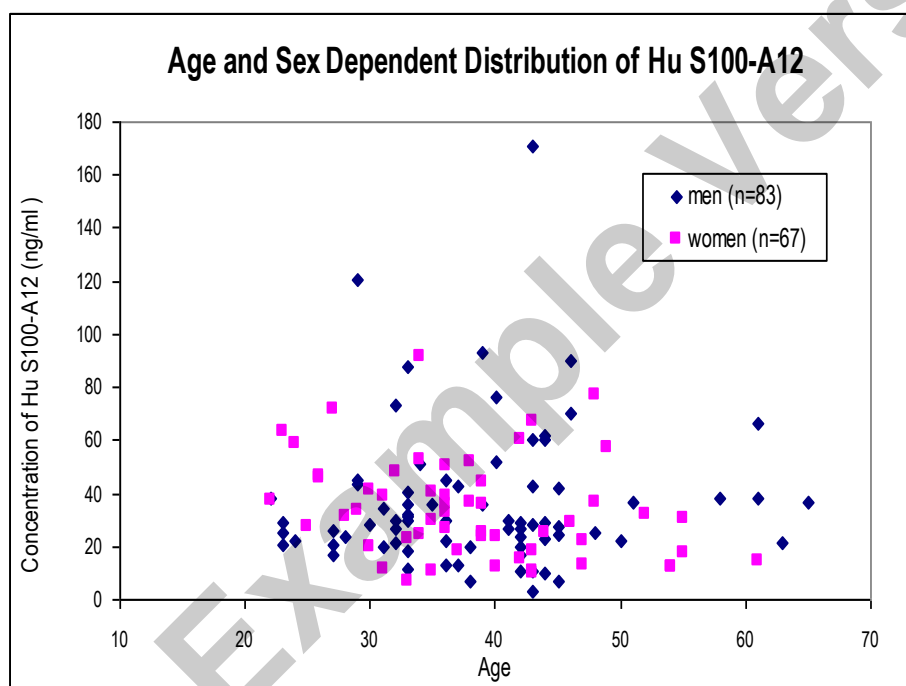


Figure 3: S100A12 concentration plotted against donor age.

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 samples in the assay. Each laboratory should establish its own normal and pathological references ranges for S100A12 levels with the assay.

16. METHOD COMPARISON

The BioVendor Human S100A12 ELISA was compared to two other immunoassays. The following correlation graphs were obtained.

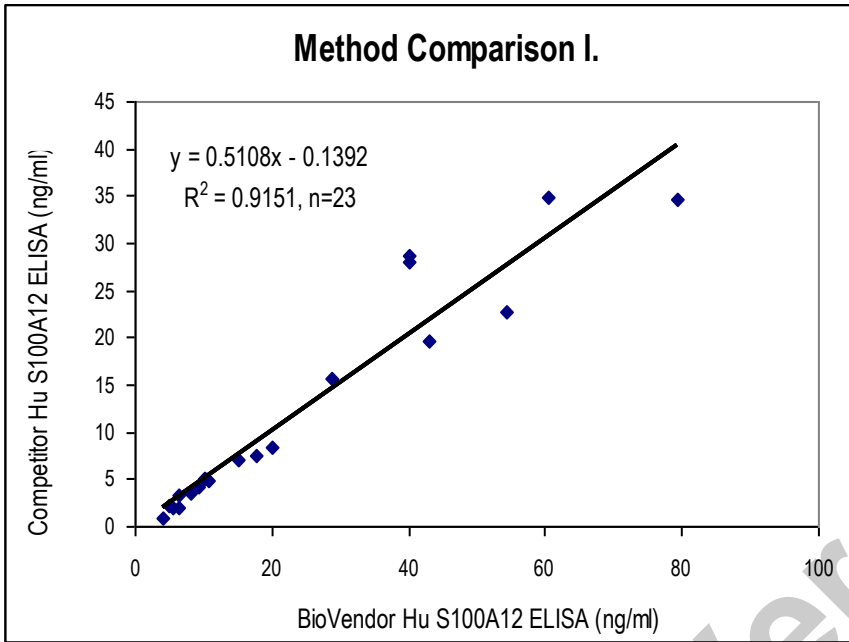


Figure 4: Method comparison I, comparison with another commercial immunoassay.

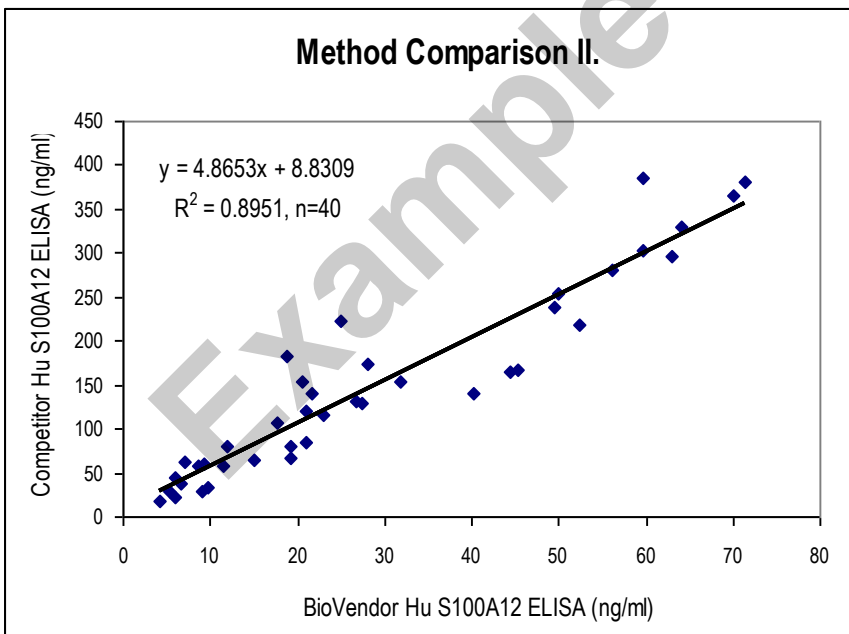


Figure 5: Method comparison II, comparison with an immunoassay independently developed by research institution.

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

High coefficient of variation (CV)

Possible explanation:

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

Example Version





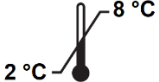




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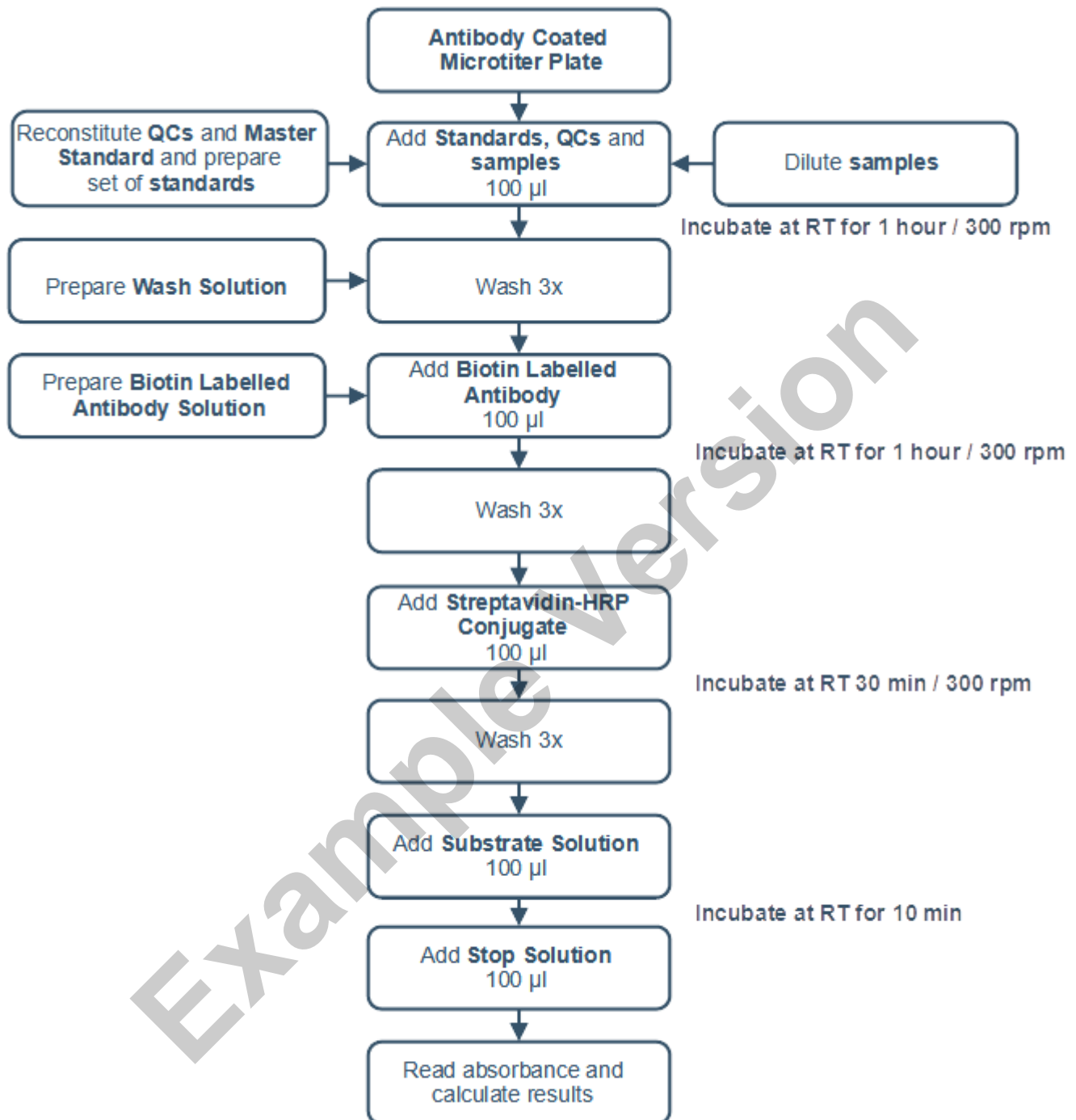
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19. EXPLANATION OF SYMBOLS

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20. ASSAY PROCEDURE - SUMMARY





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