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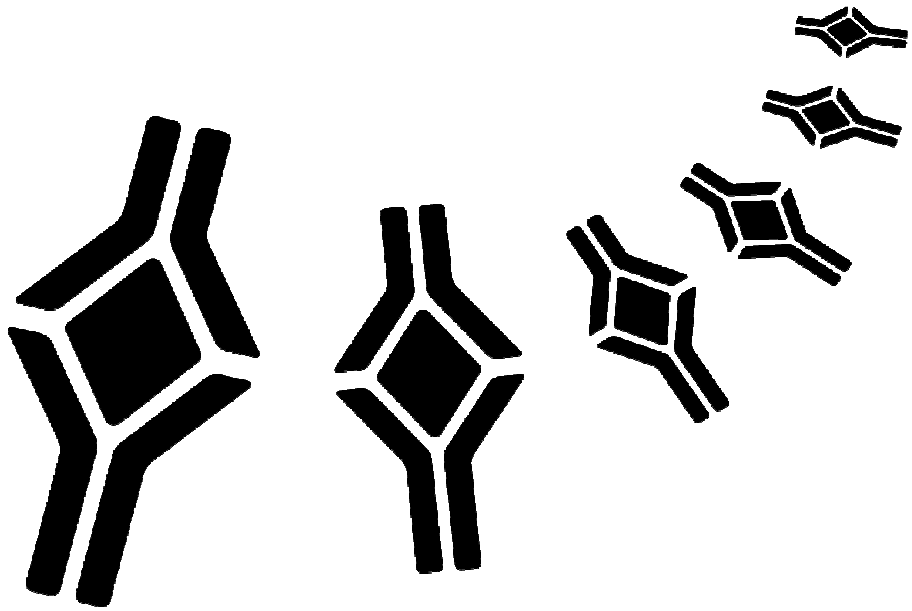

HUMAN S100A6 ELISA (Multispecies specificity)

Product Data Sheet

Cat. No.: RD191215200R

For Research Use Only

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» **This kit is manufactured by:**
BioVendor – Laboratorní medicína a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

The RD191215200R Human S100A6 ELISA (Multispecies specificity) is a sandwich enzyme immunoassay for the quantitative measurement of human and multispecies mammalian S100A6.

Features

- **It is intended for research use only**
- The total assay time is less than 3.5 hours
- The kit measures total S100A6 in human serum, heparin plasma, bronchoalveolar lavage fluid (BALF), cerebrospinal fluid (CSF), urine samples and tissue extract
- The kit measures total S100A6 in serum of several mammalian species such as cat, dog, hamster, monkey, mouse, pig, rabbit and rat
- 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

Place the lyophilized Master Standards at -20°C after the kit delivery.
Store the other kit components 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

S100A6, also known as calcyclin, is member of the S100 family of Ca^{2+}-binding proteins. Similar to other members of this family, S100A6 contains two EF-hand motifs that bind calcium ions. The S100A6 protein, similar to other S100 proteins, forms homodimers and each subunit binds two calcium ions. Ca^{2+}-binding induces conformational changes in the molecule but these structural changes are modest when compared with other Ca^{2+}-sensing proteins, such as calmodulin.

S100A6 is most abundantly expressed in fibroblasts and epithelial cells and has also been found in some neurons, astrocytes, smooth muscle cells, cardiac myocytes, platelets and lymphocytes. S100A6 is predominantly a cytoplasmic protein. In the presence of calcium ions, S100A6 might also associate with cell membranes, especially the plasma membrane and the nuclear envelope. Nuclear localization of S100A6 has also been reported.

The precise biological function of S100A6 has not yet been described. It was originally suggested that S100A6 has a role in cell-cycle progression. This hypothesis was supported by the fact that S100A6 was found to be upregulated in multiple tumors. S100A6 also inhibits the interaction between the heat shock proteins (Hsp70 and Hsp90), which suggests a potential role for S100A6 in the cellular response to different stress factors. Physiological stimuli which increase S100A6 expression include platelet-derived grow factor (PDGF), epidermal growth factor (EGF), serum, retinoic acid, estrogen, palmitate, vasopressin and gastrin. S100A6 levels are also up-regulated upon stress conditions such as ischemia, mechanical force, irradiation and oxidative stress. *In vivo* studies have demonstrated that S100A6 protein levels are elevated in myocardial diseases and in many types of tumor cells.

Animal experiments have shown that mRNA and protein levels of S100A6 are up-regulated in the surviving myocardium after infarction. In patients with acute coronary syndrome were S100A6 serum levels increased and correlated with TNF-α, suggesting a close relation of S100A6 in inflammation.

Another clinical study showed that S100A6 levels were significantly elevated in sera from women with advanced stage ovarian cancer compared to those with early stage disease. The data signify that S100A6 may prove useful in detecting and/or monitoring ovarian cancer, when used in concert with other biomarkers.

<u>Areas of investigation:</u>
Carcinomas
Myocardial diseases

4. TEST PRINCIPLE

In the BioVendor Human S100A6 ELISA, standards, quality controls and samples are incubated at 37°C in microplate wells pre-coated with polyclonal anti-human S100A6 antibody. After 60 minutes incubation and washing, biotin labelled polyclonal anti-human S100A6 antibody is added and incubated at 37°C with captured S100A6 for 60 minutes. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation at 37°C 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 S100A6. 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
- 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. (50x)	concentrated	0.28 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	2x 13 ml
Wash Solution Conc. (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml
Product Data Sheet + Certificate of Analysis	-	1 pc

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 microtitrate plate after washing
- Vortex mixer
- 37°C Incubator
- 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 desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months when 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:**

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 the S100A6 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

Prepared Standards are ready to use, do not dilute them.
Stability and storage:
Do not store the Standard stock solutions and set of standards.

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. (50x)
Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Conc. (50x) with 49 parts Biotin-Ab Diluent. Example: 20 µl of Biotin Labelled Antibody Conc. (50x) + 980 µl of Biotin-Ab Diluent for 1 strip (8 wells).
Stability and storage:
Opened Biotin Labelled Antibody Conc. (50x) 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 Conc. (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 100 ml of Wash Solution Conc. (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 Conc. (10x) is stable 3 months when stored at 2-8°C.

10. PREPARATION OF SAMPLES

The kit measures human S100A6 in serum, heparin plasma, BALF, CSF, urine samples and tissue extract.

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 lipemic and hemolytic samples.

Hemolysis in samples has a significant influence on S100A6 levels. Breakage of the red blood cell's (RBC's) causes the release of internal S100A6 into the surrounding fluid, elevates serum or plasma levels and leads to false positive results. Therefore collection of samples must be performed carefully.

Serum, heparin plasma and BALF samples:
Dilute serum samples 50x with Dilution Buffer just prior to the assay, e.g. 5 µl of sample + 245 µl of Dilution Buffer for singlets and duplicates. **Mix well** (not to foam). Vortex is recommended.

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

Urine samples:
Dilute urine samples 20x with Dilution Buffer just prior to the assay, e.g. 10 µl of sample + 190 µl of Dilution Buffer for singlets and 15 µl of sample + 285 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:
Serum samples should be stored at -20°C, or preferably at -70°C for long-term storage. Urine, BALF and CSF samples should be stored at -70°C. Avoid repeated freeze/ thaw cycles.
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 human S100A6.

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 diluted Standards, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells. See *Figure 1* for example of work sheet.
2. Incubate the plate at **37°C** for **1 hours** without shaking.
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 solution into each well.
5. Incubate the plate at **37°C** for **1 hours** without shaking.
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 **37°C** for **30 minutes** without shaking.
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 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 on 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 S100A6 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**	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 2**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 1**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 0.5**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 0.25**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 0.125**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	**QC HIGH**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	**QC LOW**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

Figure 1: Example of a work sheet.

12. CALCULATIONS

Most microplate 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 S100A6 ng/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. 1 ng/ml (from standard curve) x 50 (dilution factor) = 50 ng/ml.

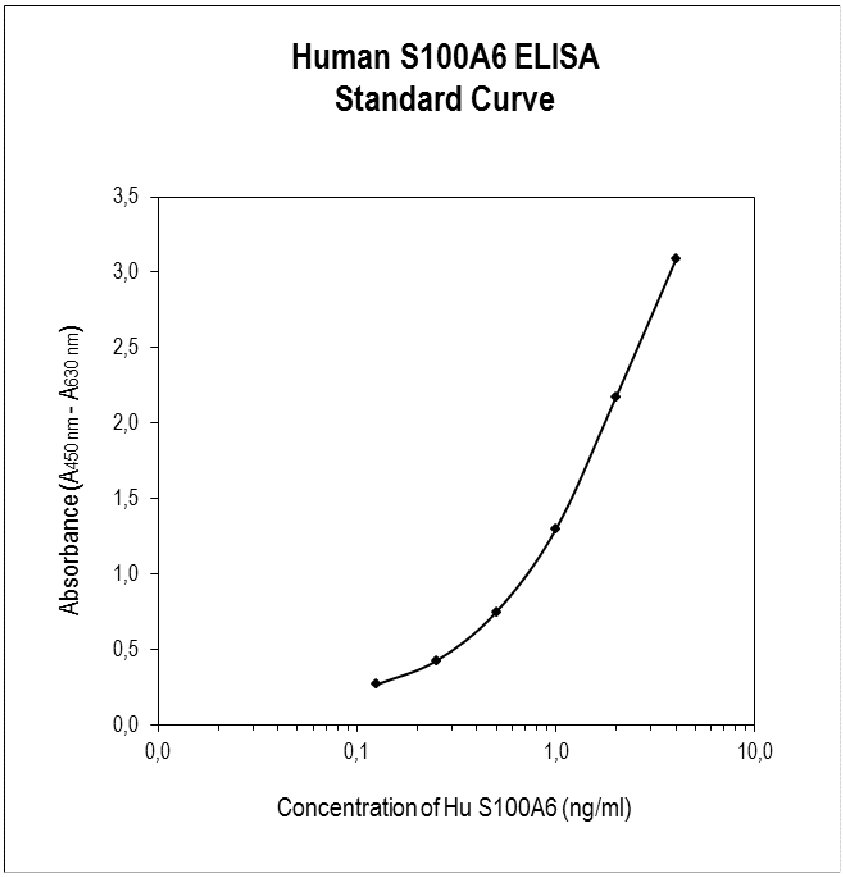

Figure 2: Typical Standard Curve for Human S100A6 ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human S100A6 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 S100A6 values in wells and is 0.014 ng/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding S100A6 level of 4 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the S100A6 concentration.

- **Specificity**

The antibodies used in this ELISA are specific for human S100A6. No crossreactivity has been observed for other human recombinant S100 proteins such as S100-A1, A4, A5, A8, A9, A10, A11, A12, A13 and S100-G protein at 200 ng/ml.
Sera of several mammalian species were measured in the assay. Species where the dilution linearity test indicated specific binding are shown below. Table contains recommended dilution for these samples.
For details please contact us at info@biovendor.com.

Mammalian serum sample	*Observed crossreactivity*	*Recommended dilution*
Bovine	no	-
Cat	yes	20x
Dog	yes	3x
Goat	no	-
Hamster	yes	6x
Horse	no	-
Monkey	yes	100x
Mouse	yes	100x
Pig	yes	3x
Rabbit	yes	40x
Rat	yes	40x
Sheep	no	-

» **Presented results are multiplied by respective dilution factor**

- **Precision**

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

Serum sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	23.84	1.38	5.8
2	33.48	1.82	5.4

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

Serum sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	25.18	1.91	7.6
2	58.78	3.30	5.6

- **Spiking Recovery**

Samples were spiked with different amounts of S100A6 and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
Serum 1	22.50	-	-
	70.65	72.50	97.4
	46.85	47.50	98.6
	32.90	35.00	94.0
Serum 2	34.35	-	-
	79.55	84.35	94.3
	57.65	59.35	97.1
	42.50	46.35	90.7
CSF 1	1.03	-	-
	3.74	4.03	92.8
	2.48	2.53	98.1
	1.73	1.78	97.3
CSF 2	2.13	-	-
	4.93	5.13	96.0
	3.63	3.63	100.1
	2.72	2.88	94.5
Urine 1	6.44	-	-
	24.00	26.44	90.8
	18.70	16.44	113.7
	13.36	11.44	116.8

Urine 2	8.12 30.92 19.02 13.46	- 28.12 18.12 13.12	- 110.0 105.0 102.6

- **Linearity**

Samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
Serum 1	- 2x 4x 8x	70.40 35.35 17.90 8.35	- 35.20 17.60 8.80	- 100.4 101.7 94.9
Serum 2	- 2x 4x 8x	100.55 50.70 26.00 13.40	- 50.30 25.15 12.55	- 100.8 103.4 106.8
CSF 1	- 2x 4x 8x	8.03 4.25 1.98 0.94	- 4.02 2.01 1.00	- 105.8 98.6 93.6
CSF 2	- 2x 4x 8x	4.83 2.36 1.22 0.56	- 2.41 1.21 0.60	- 97.8 100.9 92.0
Urine 1	- 2x 4x 8x	66.50 35.14 19.24 8.24	- 33.25 16.63 8.31	- 105.7 115.7 99.1
Urine 2	- 2x 4x 8x	25.88 12.68 6.75 3.44	- 12.54 6.47 3.24	- 98.0 103.9 106.3

- **Effect of sample matrix**

EDTA, citrate and heparin plasmas were compared to respective serum samples from the same 10 individuals.

However, we observed low correlation of S100A6 values among serum and EDTA and citrate plasma.

Results are shown below:

Volunteer No.	*Serum (ng/ml)*	*Plasma (ng/ml)*		
		EDTA	*Citrate*	*Heparin*
1	60.8	270.1	54.5	95.3
2	79.7	252.4	23.1	116.7
3	147.6	306.7	44.4	158.8
4	119.2	263.6	49.8	92.1
5	202.6	253.6	78.1	209.0
6	92.7	274.0	25.8	79.1
7	96.6	311.5	41.6	137.8
8	113.2	434.7	28.7	92.6
9	181.0	372.9	45.9	163.0
10	76.3	198.7	26.8	91.5
Mean (ng/ml)	**117.0**	**293.8**	**41.9**	**123.6**
Mean Plasma/Serum (%)	**-**	**251**	**36**	**106**
Coefficient of determination R2	**-**	**0.086**	**0.403**	**0.704**

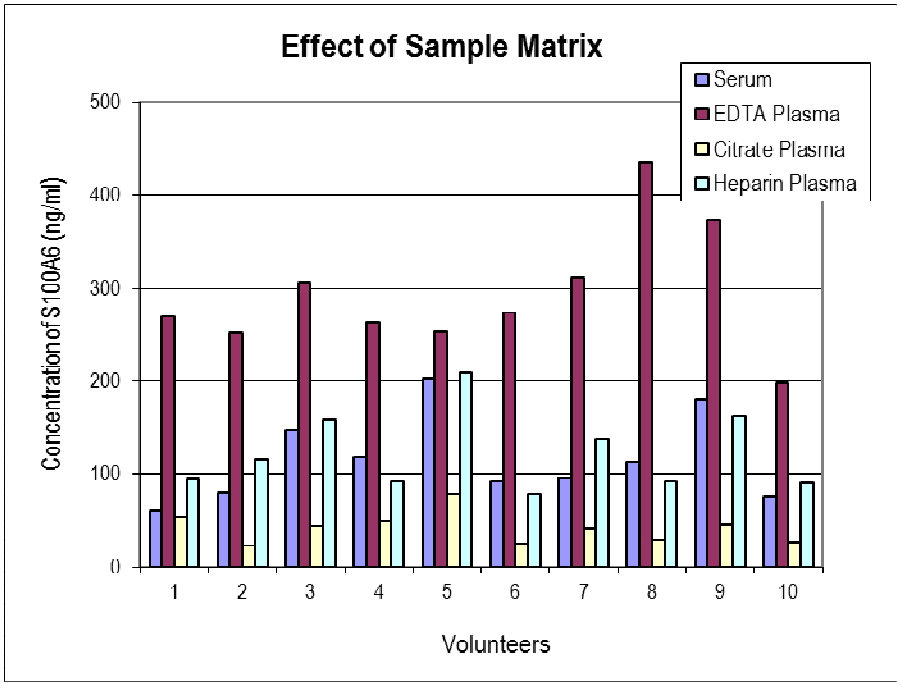

Figure 3: S100A6 levels measured using Human S100A6 ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

- **Stability of samples stored at 2-8°C**

Samples should be stored at –20°C. However, no decline in concentration of S100A6 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 thimerosal, resulting in the final concentration of 0.03% and 0.01%, respectively.

Sample	*Incubation Temp, Period*	*Serum (ng/ml)*	*Plasma (ng/ml)*		
			EDTA	*Citrate*	*Heparin*
1	-20°C	126.2	322.0	49.8	149.4
	2-8°C, 1 day	117.5	305.9	45.3	145.7
	2-8°C, 7 days	132.0	313.5	47.3	157.5
2	-20°C	113.8	289.4	49.7	148.8
	2-8°C, 1 day	107.4	273.4	47.7	155.4
	2-8°C, 7 days	103.8	279.5	37.6	134.8
3	-20°C	61.8	200.3	47.7	111.8
	2-8°C, 1 day	56.4	186.6	32.8	111.3
	2-8°C, 7 days	65.2	186.9	32.6	98.9

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human S100A6 in serum and plasma 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)*	*Plasma (ng/ml)*		
			EDTA	*Citrate*	*Heparin*
1	1x	64.7	278.5	49.5	64.6
	3x	58.5	261.1	53.8	57.2
	5x	53.4	268.3	47.0	56.8
2	1x	71.1	267.2	20.8	42.3
	3x	77.3	266.0	22.0	58.7
	5x	58.6	219.3	20.4	40.7
3	1x	77.8	235.1	32.7	63.2
	3x	72.2	225.3	40.4	62.1
	5x	64.4	235.4	36.3	71.4

14. DEFINITION OF THE STANDARD

The recombinant human S100A6 is used as the Standard. The recombinant human S100A6 produced in *E.coli*, is 11.42 kDa protein containing 100 amino acid residues of the human S100A6 and 10 extra AA.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 100 unselected donors (57 men + 43 women) 22-65 years old were assayed with the Biovendor Human S100A6 ELISA in our laboratory.

- **Age dependent distribution of S100A6**

Sex	*Age (years)*	*n*	*Mean*	*SD*	*Min.*	*Max.*
			S100A6 (ng/ml)			
Men	23-29	9	42.2	25.9	20.9	105.2
	30-39	12	36.3	18.8	14.7	86.2
	40-49	22	56.8	41.7	12.6	210.0
	50-59	4	45.0	15.5	23.4	64.4
	60-65	4	36.6	13.8	17.5	53.9
Women	22-29	9	66.3	33.8	31.9	154.4
	30-39	16	50.0	23.6	20.3	99.6
	40-49	13	53.1	34.7	21.3	138.0
	50-59	4	62.1	17.6	38.5	83.8
	60-65	1	76.5	0.0	76.5	76.5

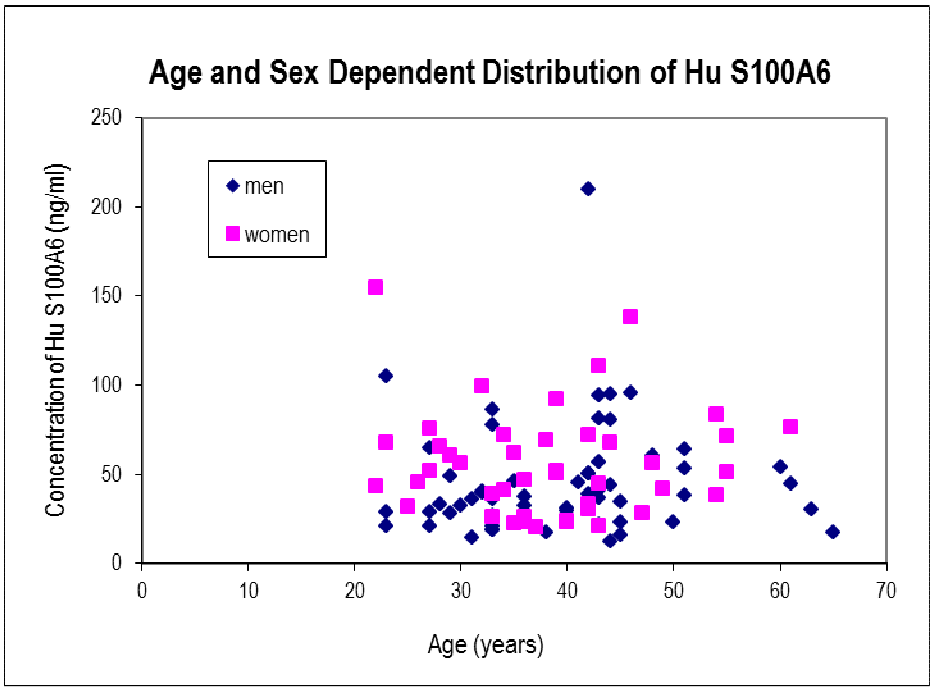

Figure 4: S100A6 concentration plotted against donor age and sex.

- **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 samples in the assay. Each laboratory should establish its own normal and pathological references ranges for S100A6 levels with the assay.

16. METHOD COMPARISON

BioVendor Human S100A6 ELISA has not been compared to any other commercial immunoassay.

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

18. REFERENCES

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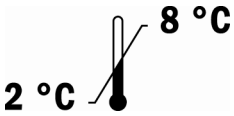
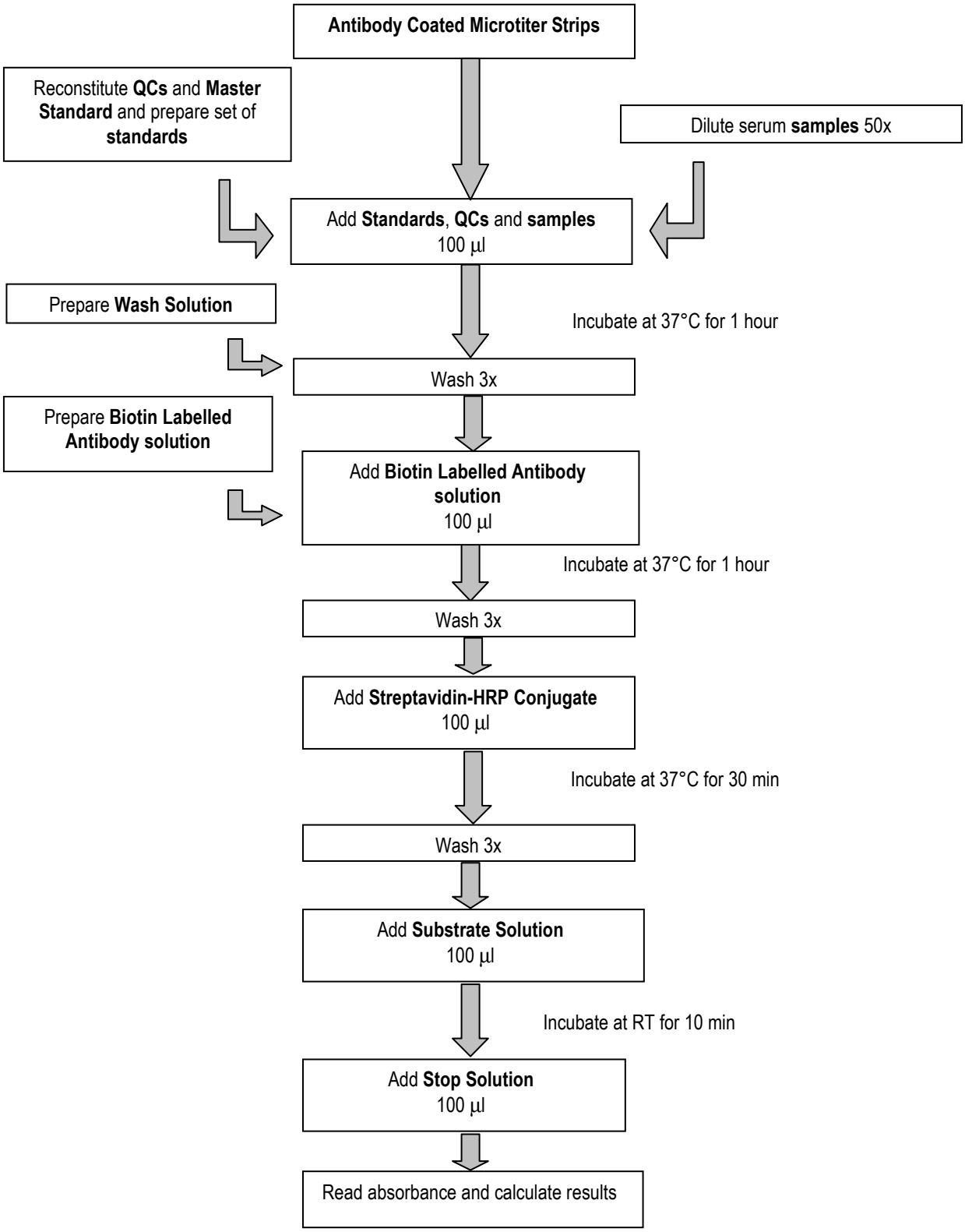
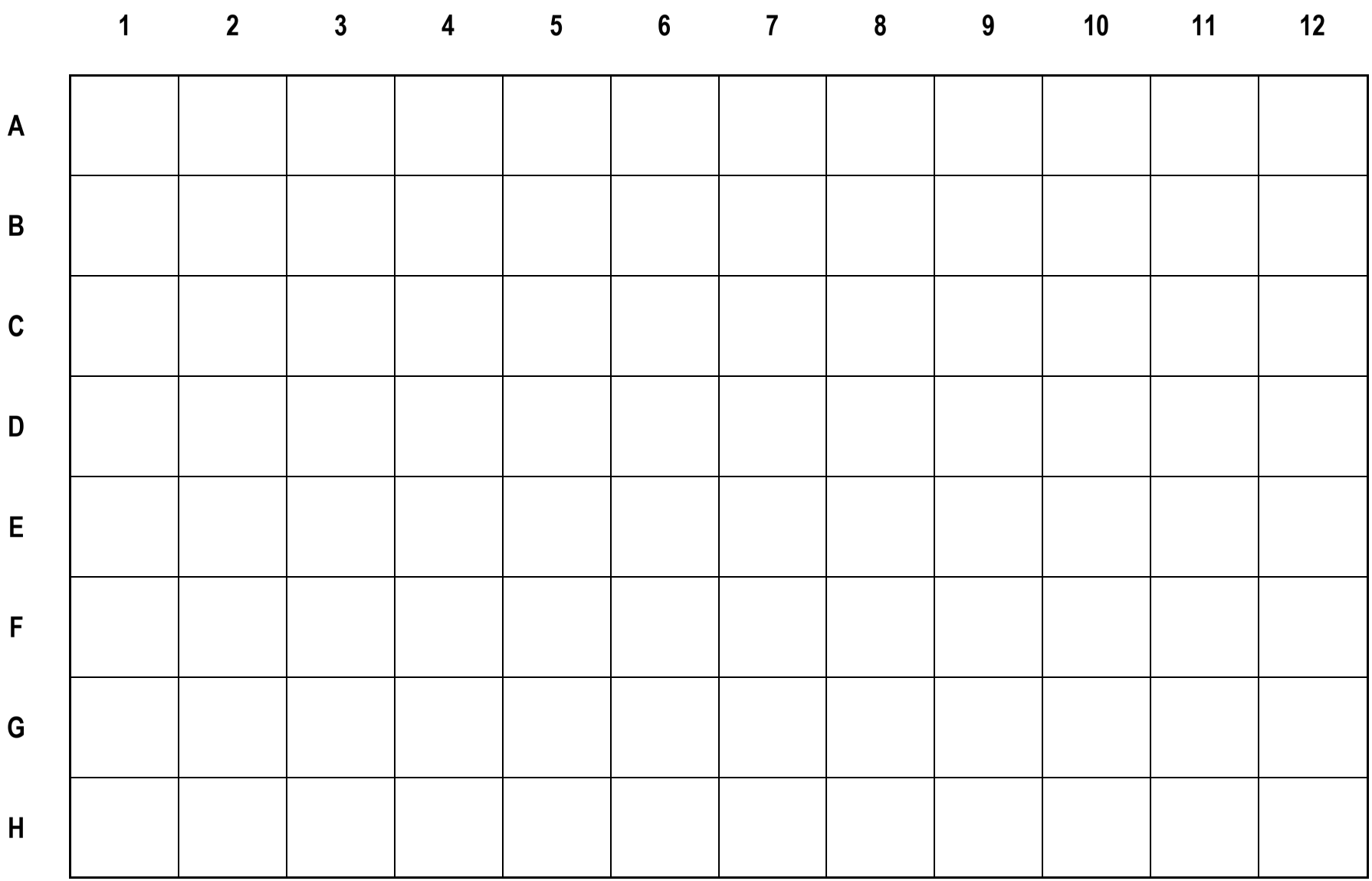
For more references on this product see our WebPages at www.biovendor.com

19. EXPLANATION OF SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	See instructions for use
	Biological hazard
	Expiry date
	Storage conditions
	Identification of packaging materials

Assay Procedure Summary

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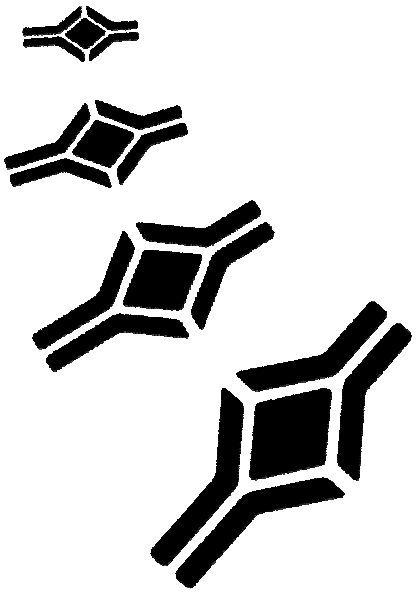
Research
and Diagnostic Products



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