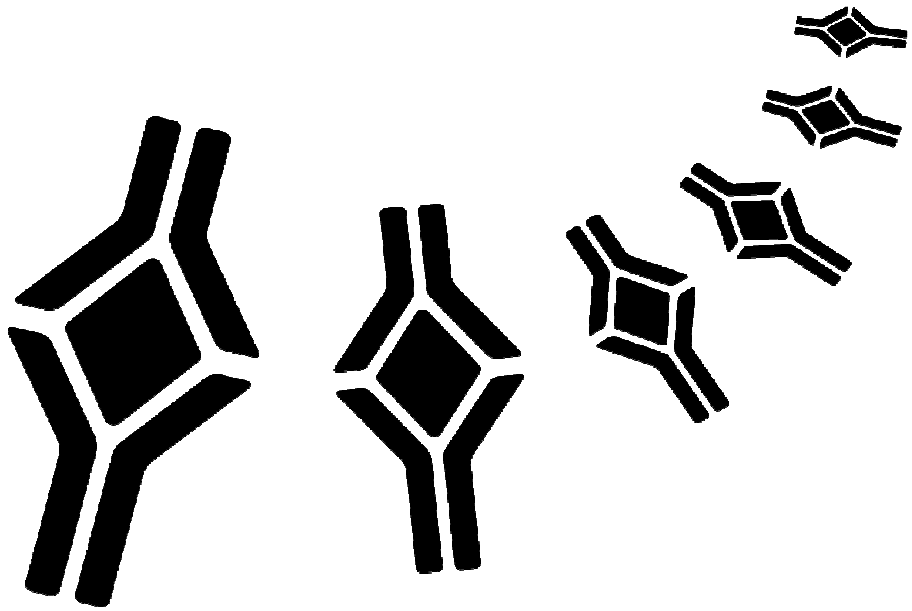
BioVendor
Research
and Diagnostic Products



HUMAN S100P ELISA

Product Data Sheet

Cat. No.: RD191048200R

For Research Use Only

CONTENTS

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 RD191048200R Human S100P ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human S100P.

Features

- **For research use only!**
- The total assay time is less than 3 hours
- The kit measures total S100P in serum and plasma (citrate, heparin)
- Assay format is 96 wells
- Standard is recombinant protein
- 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

Human S100P (also called S100 calcium-binding protein P, Protein S100-E, S100E) belongs to the S100 protein family of calcium binding protein. S100P is a 95-amino-acid protein with a molecular weight of 10.4 kDa shares 50% sequence identity with the human brain S100A1 and 44% identity with S100B.

S100P was first isolated from placenta. The placenta expresses very high levels of S100P protein in the trophoblastic cells. S100P protein has been reported to be located in the nucleus, cytoplasm, cell membrane and in the extracellular space, depending on the conditions (1, 2). S100P functions as both an extracellular and intracellular signaling molecule. In the extracellular space, S100P interacts with the receptor for advanced-glycation end products (RAGE) to activate signal transduction pathways, including the mitogen-activated protein kinase, serine protein kinase, extracellular-regulated kinase and nuclear factor pathways. Intracellular S100P interacts with the cytoskeletal multidomain protein ezrin through a Ca^{2+}-dependent mechamism (3, 4). Thus, S100P contributes to cancer progression by promoting cell proliferation, cell survival, angiogenesisand metastasis.

S100P is normally present transienty in esophageal epithelial cells during their early differentiation. The calcium-binding protein S100P is expressed in a variety of human cancer cells (pancreatic, prostate, lung, breast, colorectal and urothelial). S100P is overexpressed in human hepatocellular carcinoma (HCC). S100P expression has been noted in breast cancer, where it was associated with cellular immortalization (2, 5, 6,11,12). Higher expression of nuclear S100P (S100Pn) was typical for cases of a shorter overall survival and disease-free time. No relationship was documented between expression of S100P and sensitivity of breast cancer cells to cytostatic drugs. This study demonstrated that a high S100P expression level was associated with poor survival in early stage breast cancer patients (7).The S100P directly interacts with RAGE in pancreatic cancer cells. A RAGE antagonist peptide inhibited this interaction and also inhibited the biological effects of S100P (8). S100P may be a potential novel prognostic biomarker in colorectal cancer (CRC) patients. The patients with normal serum levels of S100P showed favourable prognosis compared with patients with elevated S100P levels (9).

S100P plays a role in the periodic change of the endometrium under the regulation of progesterone. Thus, S100P may be used as a unique biomarker of the receptive endometrium and play a role in embryo implantation (10).

Serum level of S100B, S100A6 and S100P were higher in patients with acute coronary syndrome (ACS) than in subjects with stable angina and control groups - sRAGE levels were also higher in ACS patients versus controls. S100B and S100P levels correlated significantly with CK-MB and troponin I levels in the ACS group. The results indicated that the myocardial expression of S100B, S100A6 and S100P were closely related to infarct size (13).

<u>Areas of investigation:</u>
Oncology
Cardiovascular disease
Reproduction

4. TEST PRINCIPLE

In the BioVendor Human S100P ELISA, standards and samples are incubated in a microtitrate plate wells pre-coated with polyclonal anti-human S100P antibody. After 60 min incubation and a washing, biotin-labelled polyclonal anti-human S100P antibody is added and incubated with captured S100P for 60 min. After another washing, the 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 S100P. A standard curve is constructed by plotting absorbance values against concentrations of S100P 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
- 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	lyophilized	2 vials
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Biotin-Ab Diluent	ready to use	13 ml
Dilution Buffer	ready to use	20 ml
Wash Solution Conc. (10x)	concentrate	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 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**

» **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 dessicant 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 S100P 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 (do not foam).
The resulting concentration of S100P in the stock solution is **2 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	2.0 ng/ml
300 µl of std. 2.0 ng/ml	300 µl	1.0 ng/ml
300 µl of std. 1.0 ng/ml	300 µl	0.50 ng/ml
300 µl of std. 0.50 ng/ml	300 µl	0.25 ng/ml
300 µl of std. 0.25 ng/ml	300 µl	0.125 ng/ml
300 µl of std. 0.125 ng/ml	300 µl	0.0625 ng/ml

Prepared Standards are ready to use, do not dilute them.
Stability and storage:
Reconstituted Master Standard must be used immediately.
Do not store the reconstituted and/or diluted standard solutions.

Biotin Labelled Antibody
Refer to the Certificate of Analysis for current volume of Biotin-Ab Diluent needed for reconstitution of Biotin Labelled Antibody!!!
Reconstitute the lyophilized Biotin Labelled Antibody with Biotin-Ab Diluent just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). Dilute reconstituted Biotin Labelled Antibody Concentrate (100x) with Biotin-Ab Diluent e.g. 10 µl of Biotin Labelled Antibody Concentrate + 990 µl of Biotin-Ab Diluent for 1 strip (8 wells).
Stability and storage:
Reconstituted Biotin Labelled Antibody must be used immediately.
Do not store the reconstituted and/or diluted Biotin Labelled Antibody solutions.

Wash Solution Conc. (10x)
Dilute Wash Solution Concentrate (10x) ten-fold in 900 ml of distilled water to prepare a 1x working solution, e.g. 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 S100P in serum and plasma (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.

Serum and plasma samples:
Samples should be assayed immediately after collection or should be stored at -20°C or -70°C. 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 samples.

Preparation of samples:
Dilute samples 15x with Dilution Buffer just prior to the assay e.g. 10 µl of sample + 140 µl of Dilution Buffer for singlets, or preferably 20 µl of sample + 280 µl of Dilution Buffer for duplicates.
Mix well (do not 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 effect of sample matrix (serum/plasma) on the concentration of human S100P.

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, 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 room temperature (ca. 25°C) for **1 hour**, 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 a paper towel.
4. Pipet **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. Incubation without shaking is the alternative that requires to extend incubation with substrate – see point 11.
6. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against a paper towel.
7. Pipet **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 a 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 less than 20°C. Do not shake with 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 S100P 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 2.0**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
B	**Standard 1.0**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
C	**Standard 0.5**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
D	**Standard 0.25**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
E	**Standard 0.125**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
F	**Standard 0.0625**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39
G	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40
H	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33	Sample 41

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 S100P (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. 0.45 ng/ml (from standard curve) x 15 (dilution factor) = 6.75 ng/ml.

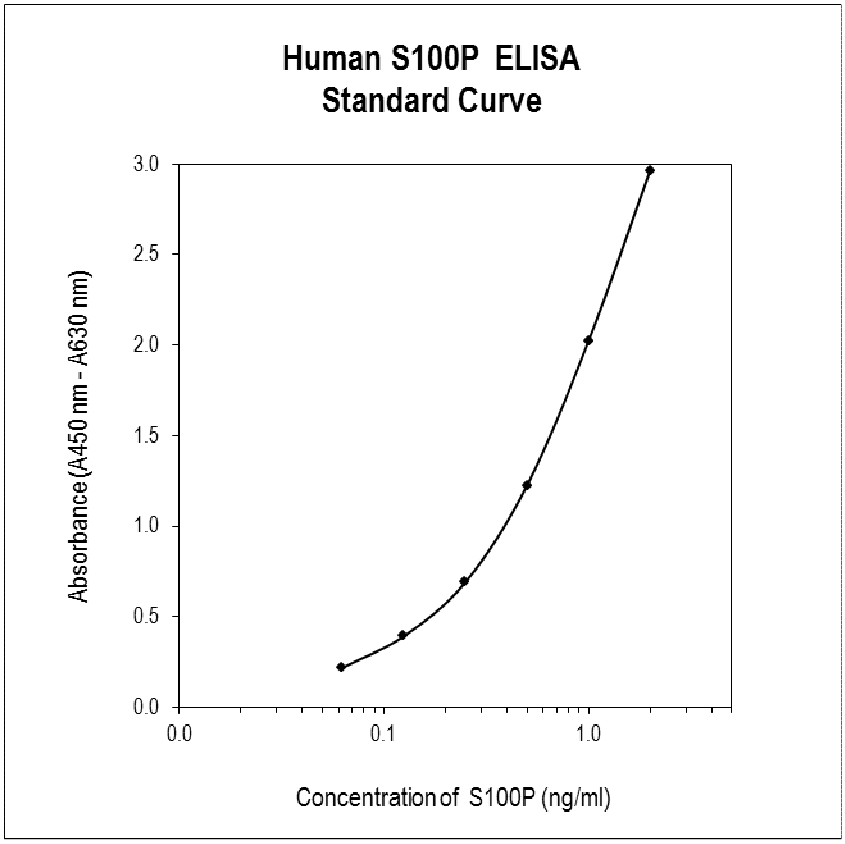


Figure 2: Typical Standard Curve for Human S100P ELISA.

13. PERFORMANCE CHARACTERISTICS

» Typical analytical data of BioVendor Human S100P 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 S100P values in wells and is 0.008 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 S100P with no detectable crossreactivities to other S100 family member (S100-A1, S100-A4, S100-A5, S100-A6, S100-A8, S100-A9, S100-A10, S100-A11, S100-A12, S100-A13, S100-A14, S100-A15, S100-A16, S100-B a S100-G) at 10 ng/ml.

» Presented results are multiplied by respective dilution factor

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	2.63	0.06	2.2
2	7.92	0.14	1.8

Inter-assay (Run-to-Run) (n=5)

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	5.91	0.41	6.9
2	8.96	0.35	3.9

- **Spiking Recovery**

Serum samples were spiked with different amounts of human S100P and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	2.73 6.27 3.92 3.66	- 6.48 4.61 3.67	- 96.8 84.9 99.7
2	3.33 11.10 7.09 5.33	- 10.33 7.08 5.20	- 102.5 100.2 102.5

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	- 2x 4x 8x	8.18 4.15 1.95 0.90	- 4.09 2.05 1.02	- 101.4 95.5 88.0
2	- 2x 4x 8x	11.90 6.14 3.01 1.43	- 5.95 2.98 1.49	- 103.1 101.2 111.0

- **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 (ng/ml)*	*Plasma (ng/ml)*		
		EDTA	*Citrate*	*Heparin*
1	4.38	9.21	2.94	3.00
2	1.21	2.84	1.49	2.33
3	0.98	2.99	1.57	1.90
4	4.06	6.64	3.51	3.61
5	3.61	8.60	2.28	2.69
6	2.56	2.96	2.81	2.38
7	1.73	1.68	1.78	1.81
8	1.41	3.88	1.68	2.00
9	2.15	3.54	2.10	2.29
10	3.86	2.55	1.62	2.13
Mean (µg/ml)	**2.59**	**4.49**	**2.18**	**2.41**
Mean Plasma/Serum (%)	**-**	173.0%	84.0%	93.0%

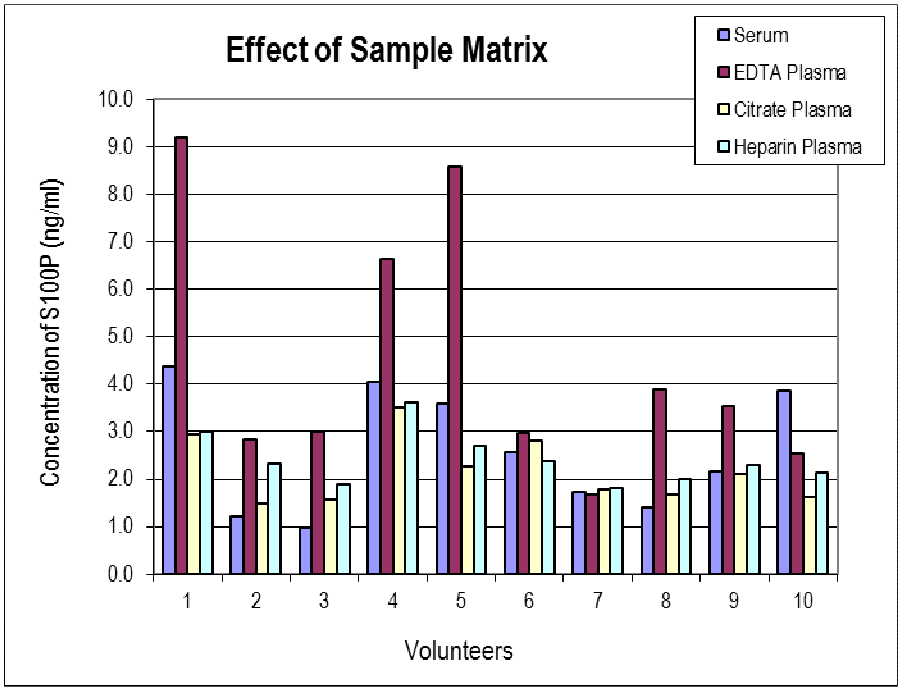


Figure 3: S100P levels measured using Human S100P ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

14. DEFINITION OF THE STANDARD

The Standard used in this kit is recombinant protein. Recombinant human S100P, produced on E. Coli, is 11.64 kDa protein consisting of 95 amino-acid residues of human S100P and 10 additional amino-acids.

15. PRELIMINARY POPULATION DATA

The following results were obtained when serum samples from 155 unselected donors (89 men + 66 women) 20 - 69 years old were assayed with the Biovendor Human S100P ELISA in our laboratory:

- **Age and Sex dependent distribution of human S100P**

Sex	*Age (years)*	*n*	*S100P (ng/ml)*				
			Mean	*Median*	*SD*	*Min*	*Max*
Men	20-29	18	4.46	4.08	2.28	1.95	12.01
	30-39	26	4.80	4.43	1.82	2.14	9.03
	40-49	31	4.59	3.81	2.16	1.45	10.61
	50-65	14	5.20	5.49	1.45	2.07	8.62
Women	20-29	12	5.38	4.75	1.96	2.72	8.79
	30-39	26	4.59	4.64	1.97	1.28	8.87
	40-49	20	4.05	3.89	1.54	1.62	6.86
	50-61	8	3.68	3.32	1.88	1.57	7.07

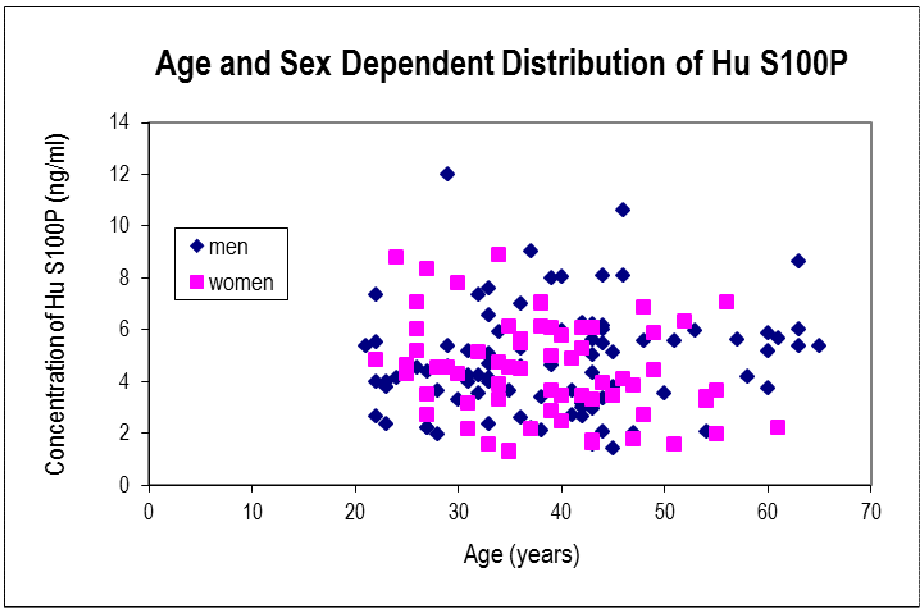


Figure 4: Human S100P concentration plotted against donor age and sex.

- **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 reference ranges for human S100P protein levels with the assay.

16. METHOD COMPARISON

The BioVendor Human S100P ELISA has not been compared to any 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
- Manual washing
- 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 or samples

›› High signal of stanbdards and samples

Possible explanations:

- Incubation temperature over 30°C. Performing the incubation at the temperature of 25°C is crucial in order to obtain valuable results!!!

18. REFERENCES

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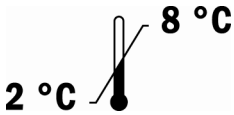
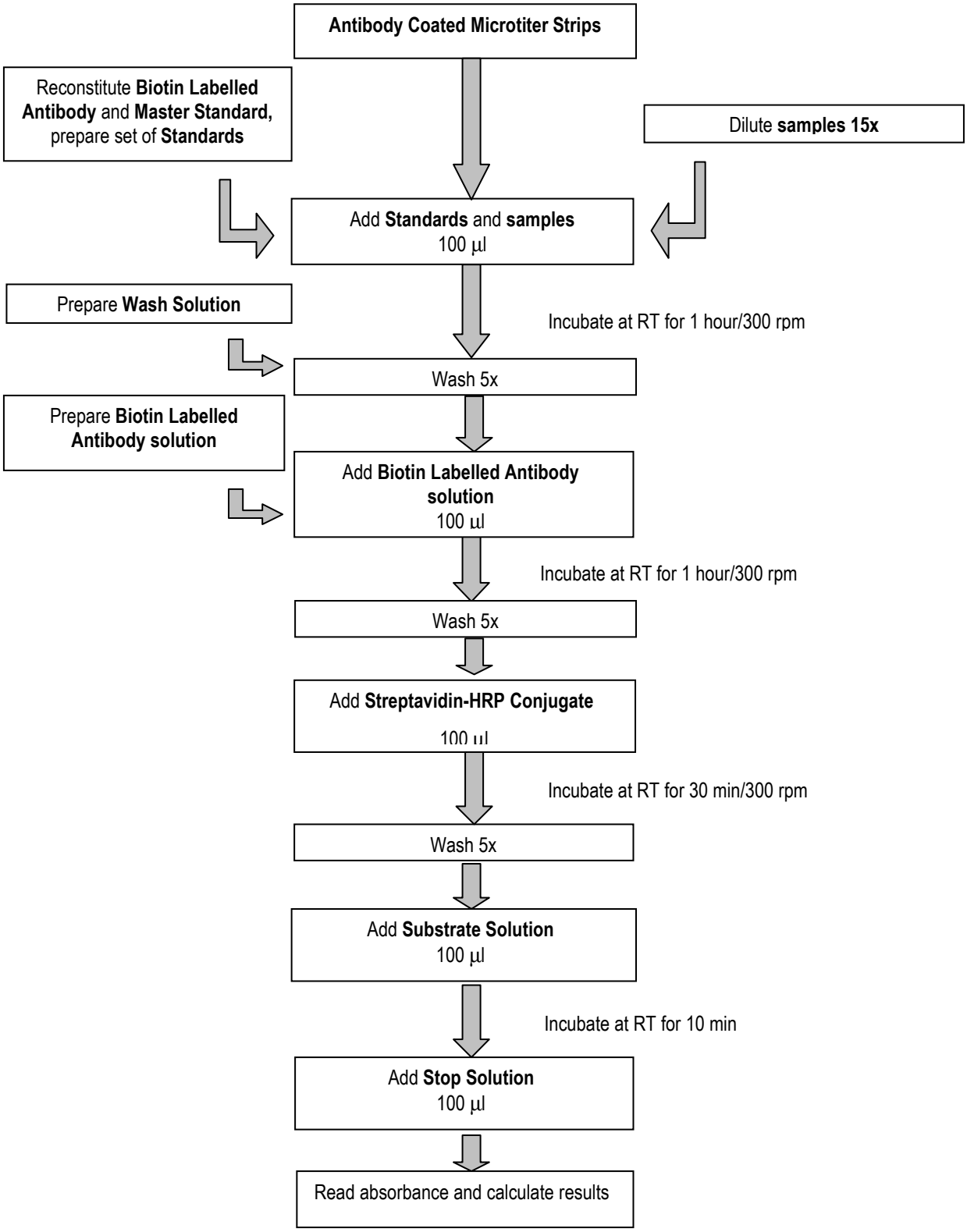
16. Shyu RY, Huang SL, Jiang SY. Retinoic acid increases expression of the calcium-binding protein S100P in human gastric cancer cells. J Biomed Sci 2003, 10 (3):313-319

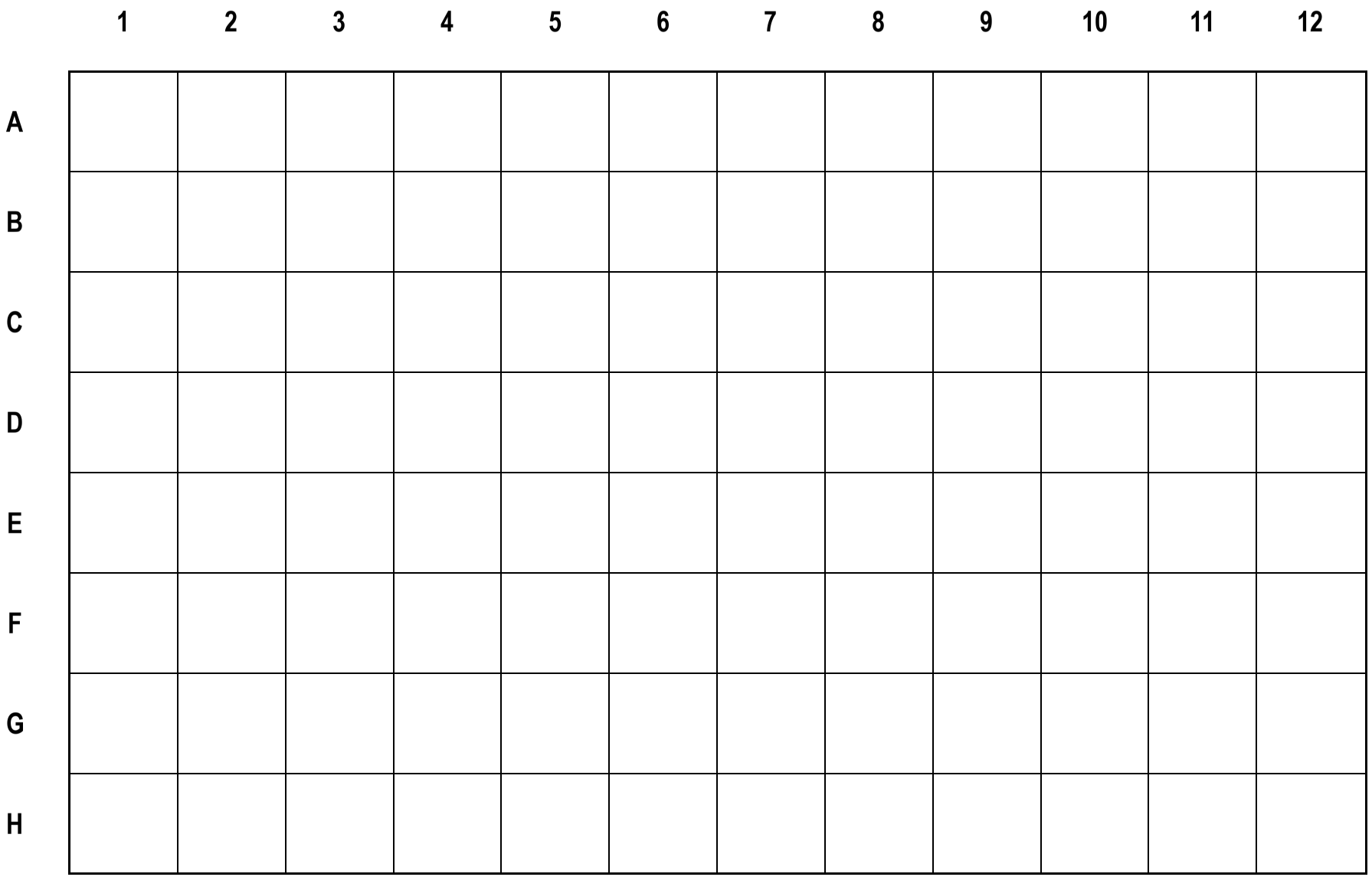
» For more references on this product see our WebPages at www.biovendor.com

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
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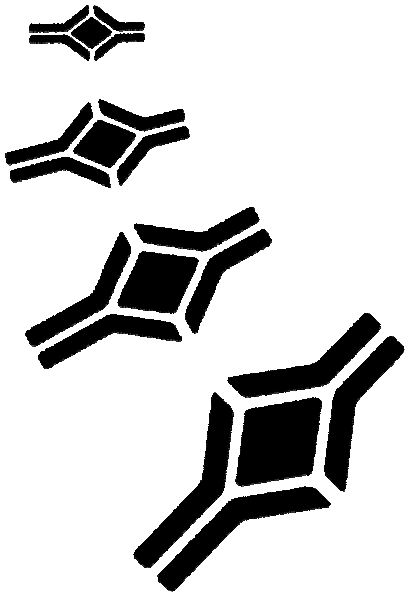

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