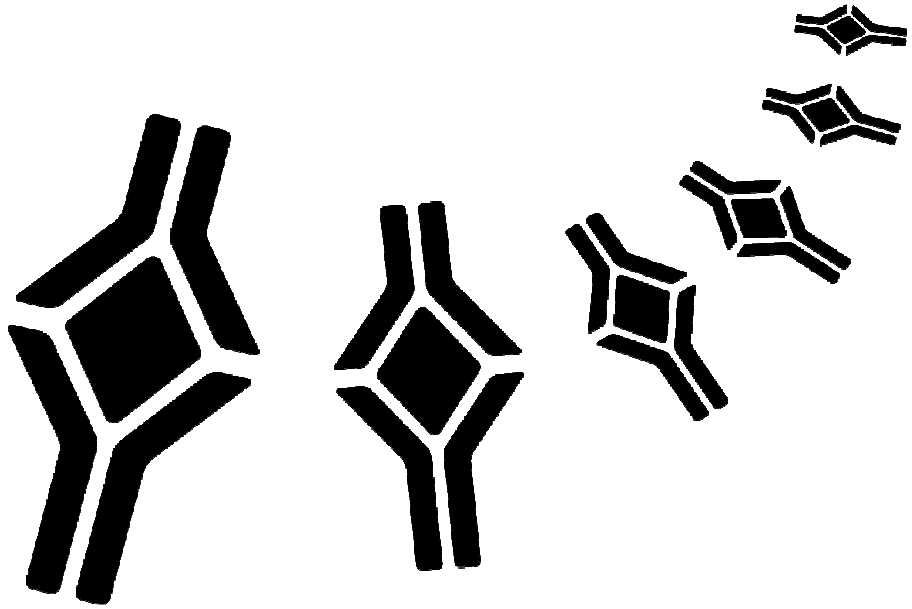
BioVendor
Research
and Diagnostic Products



HUMAN S100G ELISA

Product Data Sheet

Cat. No.: RD191225200R

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 Human S100G ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human S100G protein.

Features

- **It is intended for research use only**
- The total assay time is less than 4 hours
- The kit measures S100G in serum and plasma (EDTA, 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

S100G (S100 calcium binding protein G, Calbindin 3 (CALB3), CaBP9K, CABP1) is a member of the S100 family of calcium-binding proteins. The gene encoding human S100G is located on the X-chromosome (Xq22) and consists of three exons and contains four Alu repeats.
The S100G protein (molecular weight 9 kDa) is monomeric and contains two Ca2+ binding motifs, a classical C-terminal EF-hand with a canonical Ca2+ binding loop and an S100-specific N-terminal EF-hand with a modified Ca2+ binding loop called "pseudo EF-hand".
S100G protein is present in many organs, cartilage, bone and certain teeth, such as the ameloblasts of incisors and molars. In addition, S100G mRNA is detected in the proximal small intestine, but not in human kidney, uterus or placenta (however, the protein is present in these tissues in other species). S100G is also present in mammalian intestinal epithelial cells (enterocytes).
S100G mediates the transport of calcium across the enterocytes from the apical side, where entry is regulated by the calcium channel TRPV6, to the basolateral side, where calcium pumps such as PMCA1 utilize intracellular adenosine triphosphate to pump calcium into the blood. The transport of calcium across the enterocyte cytoplasm appears to be rate-limiting for calcium absorption in the intestine; the presence of calbindin increases the amount of calcium crossing the cell without raising the free concentration. Expression of S100G, like that of calbindin-D28k, is stimulated by the active vitamin D metabolite, calcitriol although the precise mechanisms are still controversial. In mice that lack the receptor for vitamin D, S100G is reduced, but not absent.
The members of the S100 family are multifunctional signaling proteins that influence with many cellular events. S100G appear to be involved in neurotrophic and/or neuroprotective processes, but the mechanisms of action are not completely understood. S100G has protective neurotrophic effects during brain development, and alterations in level may serve as an early, quantitative indicator of fetal brain damage in some biological fluids (eg, cord blood).
Calcium-regulatory molecule S100G is dynamically expressed and regulated in the uterine endometrium during pregnancy and suggesting that regulation of calcium ion concentration may be critical for the establishment and maintenance of pregnancy in pigs.

<u>Areas of investigation:</u>
Reproduction
Neurotrophic and/or neuroprotective processes
Intestinal Diseases

4. TEST PRINCIPLE

In the BioVendor Human S100G ELISA, the standards and samples are incubated in microtitrate wells pre-coated with polyclonal anti-human S100G antibody. After 60 minutes incubation and washing, biotin labelled polyclonal anti-human S100G antibody is added and incubated with the captured S100G for 60 minutes. After another washing, streptavidin-HRP conjugate is added. After 60 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 S100G. 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 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	lyophilized	2 vials
Streptavidin-HRP Conjugate Conc. (100x)	concentrated	0.13ml
Master Standard	lyophilized	2 vials
Dilution Buffer	ready to use	20ml
Conjugate Diluent	ready to use	2 x 13ml
Wash Solution Conc. (10x)	concentrated	100ml
Substrate Solution	ready to use	13ml
Stop Solution	ready to use	13ml
Product Data Sheet + Certificate of Analysis	-	1pc

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution
- 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
- 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
Conjugate Diluent
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 S100G 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 S100G in the stock solution is **800 pg/ml.**

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	800 pg/ml
300 µl of stock	300 µl	400 pg/ml
300 µl of 400 pg/ml	300 µl	200 pg/ml
300 µl of 200 pg/ml	300 µl	100 pg/ml
300 µl of 100 pg/ml	300 µl	50 pg/ml
300 µl of 50 pg/ml	300 µl	25 pg/ml

Prepared Standards are ready to use, do not dilute them.
Stability and storage:
The reconstituted Master Standard must be used immediately.
Do not store the Standard stock solutions and set of standards.

Biotin Labelled Antibody
Refer to the Certificate of Analysis for current volume of Conjugate Diluent needed for reconstitution of Biotin Labelled Antibody!!!
Reconstitute the lyophilized Biotin Labelled Antibody with Conjugate 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 Conjugate Diluent e.g. 10 µl of Biotin Labelled Antibody Concentrate + 990 µl of Conjugate Diluent for 1 strip (8 wells).
Stability and storage:
Biotin Labelled Antibody Concentrate (100x) is stable 1 month when stored at 2-8°C.
Do not store the diluted Biotin Labelled Antibody solution.

Streptavidin-HRP Conjugate Conc. (100x):
Prepare the working Streptavidin-HRP Conjugate solution by adding 1 part Streptavidin-HRP Conjugate Concentrate (100x) with 99 parts Conjugate Diluent. Example: 10 µl of Streptavidin-HRP Conjugate Concentrate (100x) + 990 µl of Conjugate Diluent for 1 strip (8 wells).
Stability and storage:
Opened Streptavidin-HRP Conjugate Conc. (100x) is stable 3 months when stored at 2-8°C.
Do not store the diluted Streptavidin-HRP Conjugate 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 S100G in 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.

An appropriate dilution should be assessed by the researcher (due to the large variability of serum S100G levels different individuals) in advance to batch measurement. Recommended starting dilution is 35x.

Dilute serum or plasma samples 35x with Dilution Buffer just prior to the assay, e.g. 5 µl of sample + 170 µl of Dilution Buffer when assaying samples as singlets or preferably 8 µl of sample + 272 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:
Samples should be stored at -20°, 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 and plasma samples when stored at 2 - 8° C and effect of sample matrix (serum/plasma) on the concentration of human S100G.

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, 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 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 **1 hour**, 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. (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). No shaking!
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 S100G 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 800**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
B	**Standard 400**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
C	**Standard 200**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
D	**Standard 100**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
E	**Standard 50**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
F	**Standard 25**	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 microtiter plate readers perform automatic calculations of analyte concentration. The Standards 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 S100G (pg/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. 100 pg/ml (from standard curve) x 35 (dilution factor) = 3500 pg/ml.

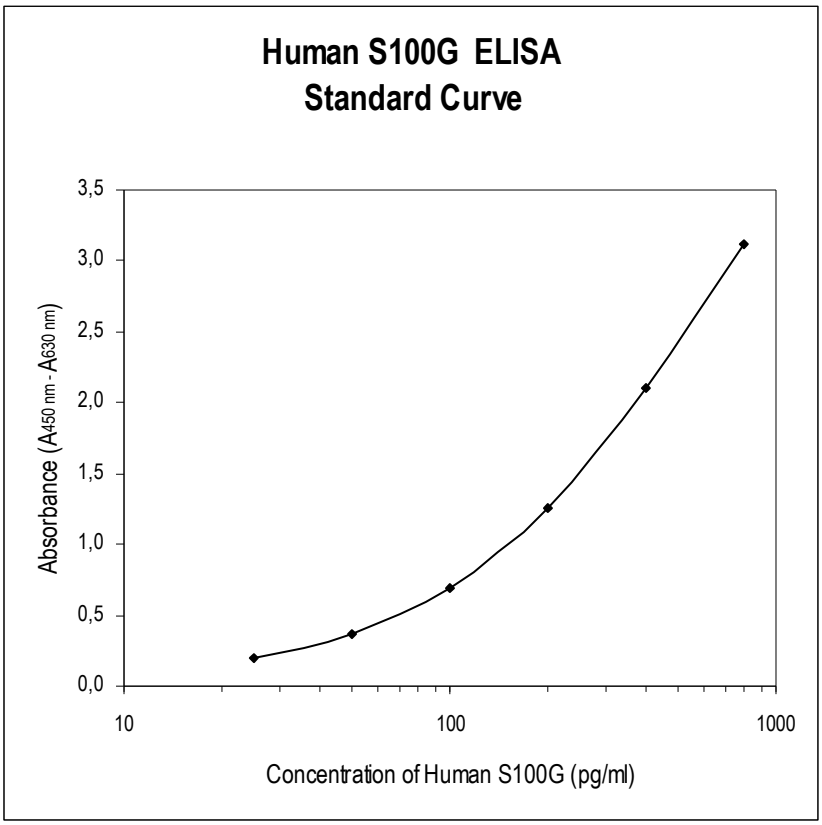


Figure 2: Typical Standard Curve for Human S100G ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human S100G 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 S100G values in wells and is 7.7 pg/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 S100G.

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	yes
Mouse	yes
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 (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	4.9	0.2	3.6
2	5.5	0.2	4.1

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	2.3	0.2	7.2
2	5.0	0.2	4.5

- **Spiking Recovery**

Serum samples were spiked with different amounts of human S100G and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	2.5	-	-
	3.2	3.4	95.9
	4.2	4.2	100.0
	5.6	6.0	94.5
2	2.1	-	-
	2.8	2.9	94.2
	3.6	3.8	94.0
	5.9	5.6	106.6

- **Linearity**

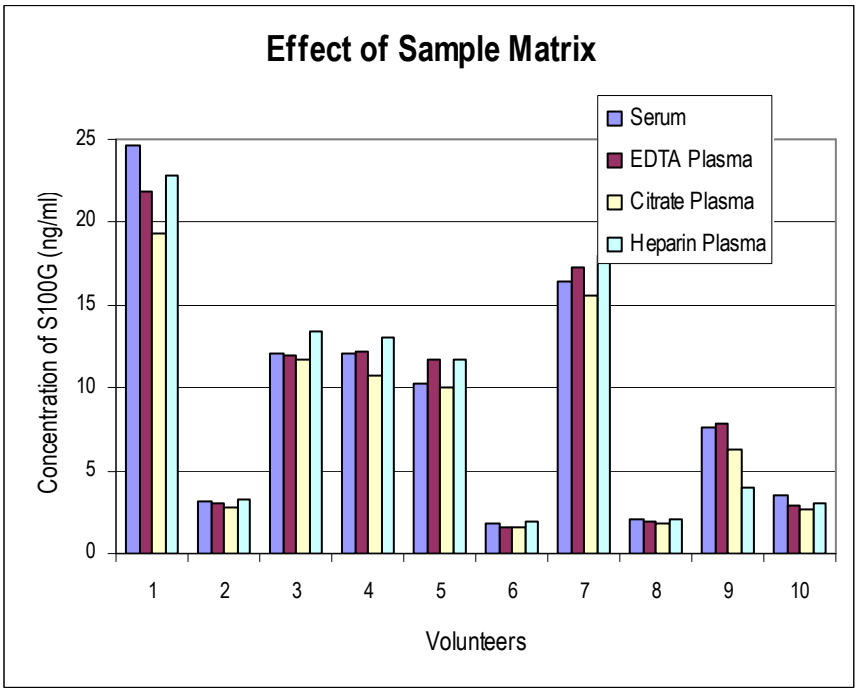
Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	-	23.0	-	-
	2x	10.8	11.5	94.3
	4x	5.3	5.7	92.1
	8x	2.7	2.9	92.6
2	-	19.7	-	-
	2x	9.3	9.9	94.1
	4x	4.8	4.9	97.1
	8x	2.5	2.5	99.9

- **Effect of sample matrix**

EDTA, citrate and heparin plasma samples 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	24.7	21.8	19.4	22.8
2	3.1	3.0	2.8	3.3
3	12.1	11.9	11.7	13.4
4	12.0	12.2	10.7	13.0
5	10.2	11.7	10.0	11.7
6	1.9	1.6	1.6	1.9
7	16.5	17.3	15.6	18.0
8	2.1	2.0	1.8	2.1
9	7.6	7.9	6.3	4.0
10	3.5	3.0	2.6	3.0
Mean (ng/ml)	**9.4**	**9.2**	**8.3**	**9.3**
Mean Plasma/Serum (%)		**98.8%**	**88.3%**	**99.5%**
Coefficient of determination R^2		**0.98**	**0.98**	**0.95**



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

Samples should be stored at -20°C or preferably at -70°C. However, no significant decline in concentration of human S100G 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	*Storage Conditions*	*Serum (ng/ml)*	*Plasma (ng/ml)*		
			EDTA	*Citrate*	*Heparin*
1	-20°C	0.9	0.8	0.7	0.9
	2-8°C, 1 day	0.9	0.8	0.7	0.9
	2-8°C, 7 days	0.8	0.7	0.7	0.8
2	-20°C	5.6	5.1	4.2	4.9
	2-8°C, 1 day	5.7	5.1	4.6	5.2
	2-8°C, 7 days	5.8	4.8	4.2	5.0
3	-20°C	0.2	0.2	0.2	0.2
	2-8°C, 1 day	0.2	0.2	0.2	0.2
	2-8°C, 7 days	0.9	0.8	0.7	0.9

- **Reference range**

The reference range of serum samples from healthy volunteers (N=137, 77 men and 60 women aged 21-65) has been determined using this Human S100G ELISA kit in our laboratory: Normal range comprised S100G mean concentration 3.0 ng/ml (SD 6.2).
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 S100G levels with the assay.

14. DEFINITION OF THE STANDARD

The recombinant human S100G is used as the Standard. The recombinant human S100G, produced in *E. coli*, is 10.04 kDa protein containing 87 amino acid residues of the human S100G and 9 additional amino acid residues- His Tag.

15. 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 or samples

16. REFERENCES

References to S100G:

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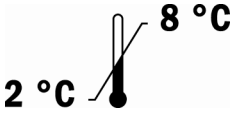
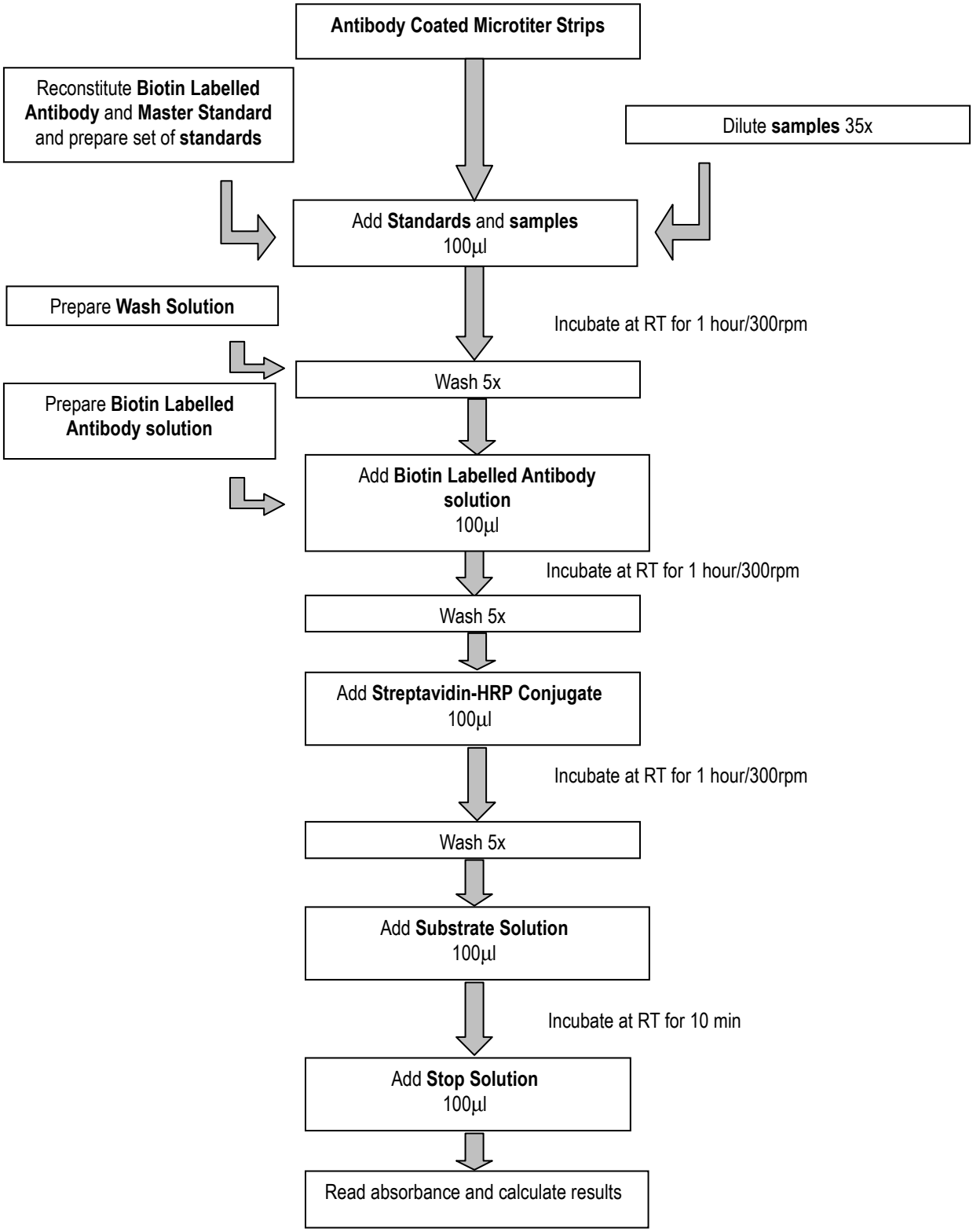
- Griffiths EJ, Rutter GA. Mitochondrial calcium as a key regulator of mitochondrial ATP production in mammalian cells. Biochimica et Biophysica Acta 1787 (2009) 1324–1333. Epub 2009 February 3.
- Donato R. RAGE: A Single Receptor for Several Ligands and Different Cellular Responses: The Case of Certain S100 Proteins. Current Molecular Medicine 2007, 7, 711-724.

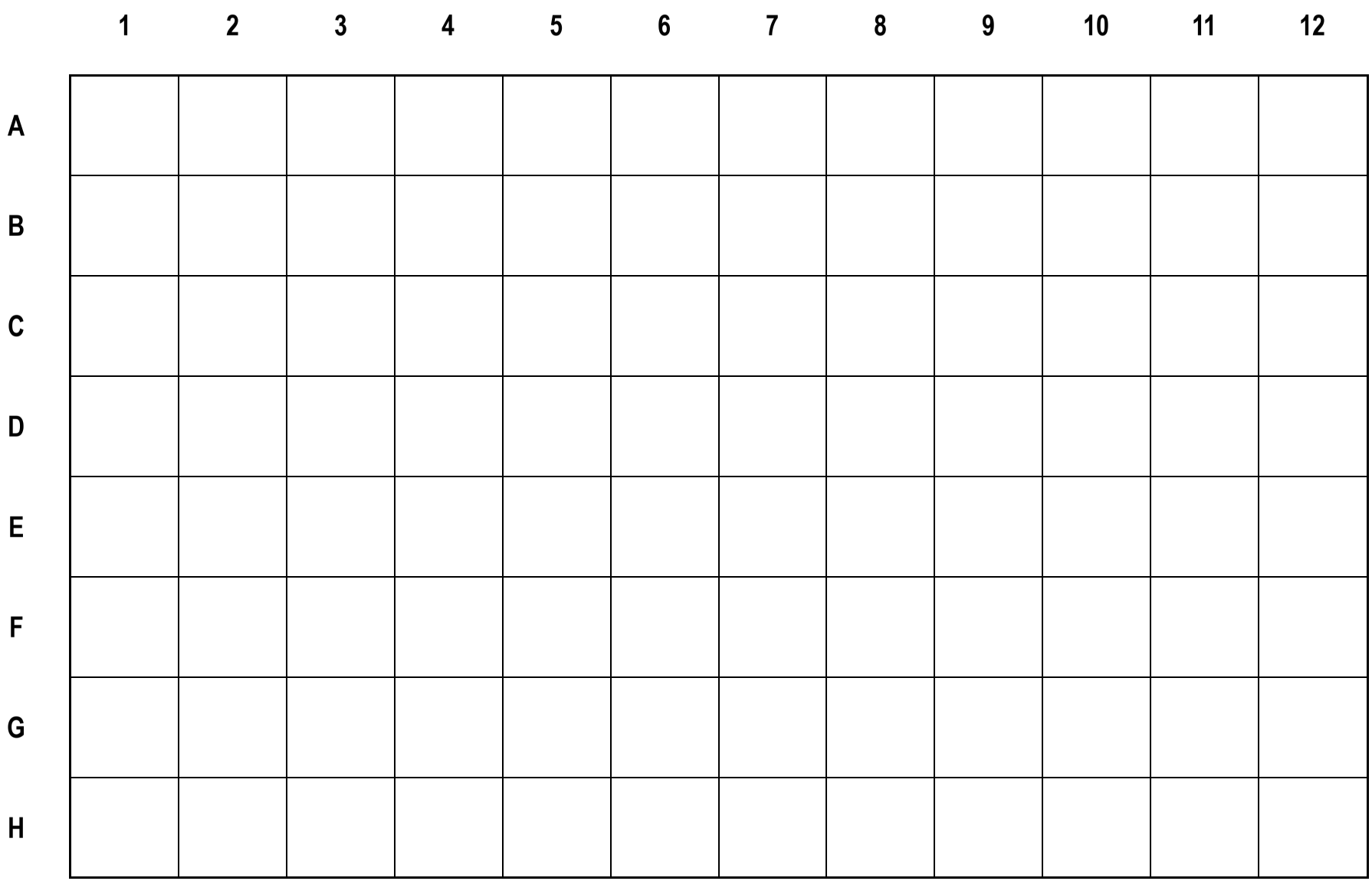
For more references on this product see our WebPages at www.biovendor.com

17. EXPLANATION OF SYMBOLS

	Catalogue number
	Content
	Lot number
	See instructions for use
	Expiry date
	Storage conditions
	Identification of packaging materials

Assay Procedure Summary

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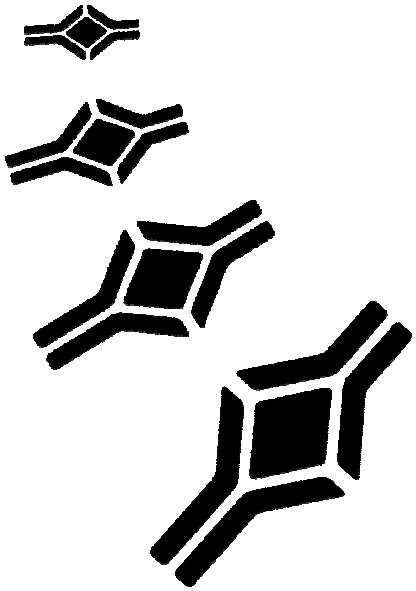

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