BioVendor Group

ENG

Instructions for Use:
HUMAN S100B ELISA

Catalogue number:
RD192090100R

For research use only!

BioVendor R&D

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CONTENT

HISTORY OF CHANGES

Previous version	Current version
ENG.008.A	ENG.009.A
A symbol indicating the manufacturer added.	

1. INTENDED USE

The RD192090100R Human S100B ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human S100B.

Features

- **It is intended for research use only**
- The total assay time is less than 4.5 hours
- The kit measures S100B protein in serum and cerebrospinal fluid samples
- Assay format is 96 wells
- Quality Controls are human serum based. Animal serum is used for Master Standard and Dilution Buffer preparations
- Standard is native 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

S-100B is a member of highly homologous Ca^{2+} binding proteins family that possess two EF-hand motifs. The family of S100 proteins consists of 19 members. Most S100 proteins exist as dimers (frequently homodimers) within cells. Exclusively expressed in vertebrates, S100 is implicated in various intracellular and extracellular regulatory activities. Studies indicate that S100 proteins are involved in the inhibition of protein phosphorylation, inhibition of cytoskeletal constituent assembly, regulation of Ca^{2+} homeostasis, stimulation of enzyme activities, and interaction with transcription factors. S100B is abundant in the nervous system where it is predominantly expressed in astrocytes, oligodendrocytes and Schwann cells. When secreted by astrocytes, S100B has neurotrophic effects during development and nerve regeneration at physiologic (nanomolar) concentrations. However, high (micromolar) concentrations of S100B have shown to be neurotoxic, participating in the physiology of neurodegenerative disorders. The clinical values have been demonstrated in stroke, cerebral complications associated with cardiac arrest and in patients with severe as well as minor head injuries. Patients with progressive melanoma disease also show elevated serum concentrations of S100B.

Areas of investigation:
Neural tissue damage
Acute myocardial infarction
Oncology

4. TEST PRINCIPLE

In the BioVendor Human S100B ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with monoclonal anti-human S100B antibody. After 120 minutes incubation and washing, biotin labelled monoclonal anti-human S100B antibody is added to the wells and incubated for 60 minutes with captured S100B. After another washing, Streptavidin-HRP Conjugate is added. After 30 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the Substrate Solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of S100B. 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. (100x)	concentrated	0.13 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vial
Quality Control HIGH	lyophilized	1 vial
Quality Control LOW	lyophilized	1 vial
Biotin-Ab Diluent	ready to use	13 ml
Dilution Buffer	ready to use	20 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
- Precision pipettes to deliver 10-1000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
- Vortex mixer
- Orbital microplate shaker capable of approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550 - 650 nm)
- Software package facilitating data generation and analysis (optional)

9. PREPARATION OF REAGENTS

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

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 S100B Master Standard

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!
Reconstitute the lyophilized Master Standard according to the Certificate of Analysis to prepare standard stock solution just prior to the assay. Let it dissolve for 25-30 minutes with occasional gentle shaking (not to foam). The resulting concentration of the human S100B in the stock solution is **1600 pg/ml**.

Prepare set of standards using **Dilution Buffer** as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	-	1600 pg/ml
100 µl of stock	400 µl	320 pg/ml
250 µl of 320 pg/ml	250 µl	160 pg/ml
250 µl of 160 pg/ml	250 µl	80 pg/ml
250 µl of 80 pg/ml	250 µl	40 pg/ml
250 µl of 40 pg/ml	250 µl	20 pg/ml
250 µl of 20 pg/ml	250 µl	10 pg/ml

Mix thoroughly but gently (not to foam) after each dilution step. 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 distilled water needed for reconstitution and for current Quality Control concentration!!!
Reconstitute each Quality Control (HIGH and LOW) with distilled water just prior to the assay. Let it dissolve for 25-30 minutes with occasional gentle shaking (not to foam).
Dilute Quality Controls prior to the assay 4x with Dilution Buffer, e.g. 40 µl of Quality Control + 120 µl of Dilution Buffer for singlets, or preferably 60 µl of Quality Control + 180 µl of Dilution Buffer for duplicates.

Stability and storage:
The reconstituted Quality Controls must be used immediately or aliquoted and frozen at -20°C for 3 months. Avoid repeated freeze/thaw cycles.
Do not store the diluted 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 IFU 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 of Biotin Labelled Antibody Conc. (100x) to 99 parts of Biotin-Ab Diluent.
Example (for 1 strip, i.e. 8 wells): 10 µl of Biotin Labelled Antibody Conc. (100x) + 990 µl Biotin-Ab Diluent.

Stability and storage:
Opened Biotin Labelled Antibody Conc. (100x) is stable 3 months when stored at 2-8°C.
Do not store 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 S100B in serum and cerebrospinal fluid (CSF) samples. EDTA plasma and citrate plasma are not suitable kinds of samples because of unfavourable interactions between sample and Dilution Buffer components. No similar interaction has been observed with heparin plasma samples therefore it can be assumed that heparin plasma samples may be used in this ELISA. However heparin plasma samples could not be validated with this ELISA because suitable samples paired with serum samples from the same individuals were not available.

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

An appropriate sample dilution should be assessed by the researcher in advance to batch measurement.

Recommended starting dilution for serum and CSF samples is 4x.
Dilute samples 4x with Dilution Buffer just prior to the assay, e.g. 40 µl of sample + 120 µl of Dilution Buffer for singlets, or preferably 60 µl of sample + 180 µl of Dilution Buffer for duplicates. **Mix thoroughly but gently** (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 cerebrospinal fluid sample when stored at 2-8°C and effect of freezing/thawing on the concentration of S100B protein.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.
Ask for more detailed information at info@biovendor.com if assaying heparin plasma samples or other kinds of samples.

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 room temperature (ca. 25°C) for **120 minutes**, 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 **60 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add **100 µl** of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for **30 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
10. Add **100 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
11. Incubate the plate for **15 - 30 minutes** at room temperature. Longer incubation time (up to 30 minutes) is recommended if the colour development is slow. 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 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 S100B 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 5-times: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat 4-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 320**	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 160**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 80**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 40**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 20**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 10**	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 S100B pg/ml in samples.

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

The measured concentration of S100B in samples and in Quality Controls calculated from the standard curve must be multiplied by their respective dilution factor, because samples and controls have been diluted prior to the assay, e.g. 75.5 pg/ml (from standard curve) x 4 (dilution factor) = 302 pg/ml.

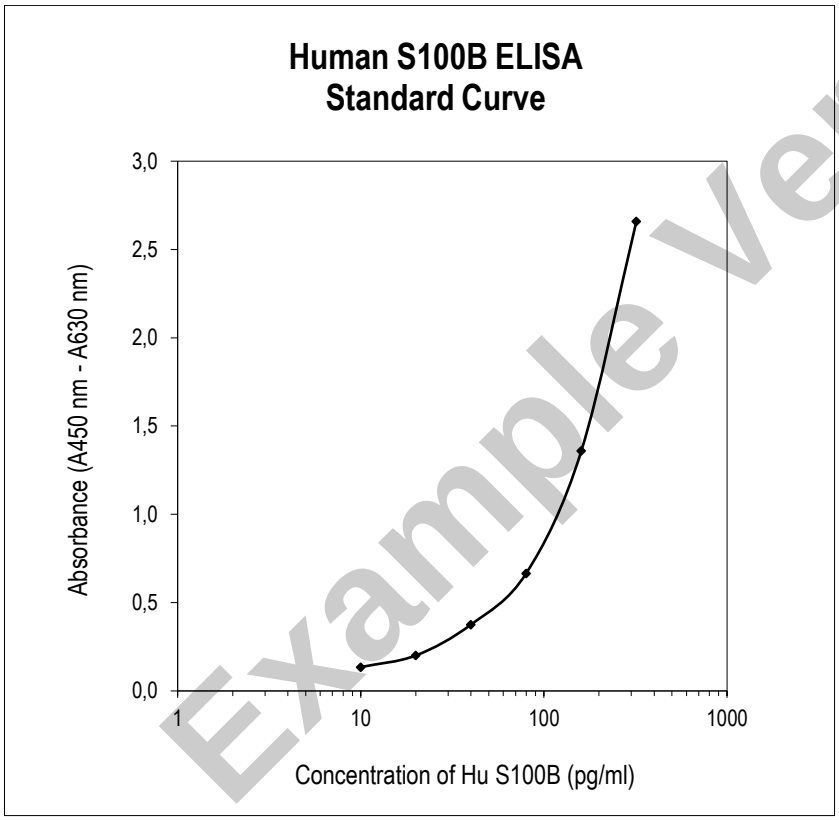

Figure 2: Typical Standard curve for Human S100B ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human S100B ELISA are presented in this chapter.

Sensitivity

The measurement range of the assay is 10 - 320 pg/ml (final concentration in a well after dilution).

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 S100B with no detectable crossreactivities to human S100A1, S100A4, S100A5, S100A8, S100A9, S100A10, S100A11, S100A14, S100A15, S100A16, S100G.

High S100B concentration was detected in diluted (1 000x – 2 000x) mouse brain homogenate samples using this ELISA.

Sera of several mammalian species were measured in the assay. Positive reactivity has been found in these animal sera: bovine, cat, goat, hamster, rhesus monkey, rabbit and rat.
For details please contact us at info@biovendor.com.

S100B protein amino acid sequence is highly conserved (e.g. there is only 1 amino acid difference between mouse and human S100B protein sequences), therefore cross reactivity with some animal S100B proteins may be expected. On the other hand, very low or zero S100B levels found in animal serum samples can reflect in some cases only absence of S100B in those particular serum samples from animals with the uninjured brain.

Presented results are multiplied by respective dilution factor.

Precision

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

Sample	Mean (pg/ml)	SD (pg/ml)	CV (%)
1	259.3	5.4	2.1
2	403.4	7.8	1.9

Inter-assay (Run-to-Run) (n=4)

Sample	Mean (pg/ml)	SD (pg/ml)	CV (%)
1	105.0	7.5	7.1
2	341.3	16.0	4.7

Spiking Recovery

Serum and cerebrospinal fluid (CSF) samples were spiked with different amounts of human S100B antigen and assayed.

Sample	Observed (pg/ml)	Expected (pg/ml)	Recovery O/E (%)
Serum sample	568.6	-	-
	863.8	888.6	97.2
	1168.9	1208.6	96.7
	1760.8	1848.6	95.3
CSF sample	268.2	-	-
	424.4	368.2	115.3
	478.1	468.2	102.1
	706.4	668.2	105.7

Linearity

Serum and CSF samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (pg/ml)	Expected (pg/ml)	Recovery O/E (%)
Serum sample	-	420.5	-	-
	2x	212.3	210.3	101.0
	4x	111.2	105.1	105.7
	8x	58.6	52.6	111.4
CSF sample	-	827.3	-	-
	2x	481.3	413.7	116.4
	4x	237.1	206.8	114.6
	8x	124.7	103.4	120.6

Stability of samples stored at 2-8°C

Samples should be stored at –20°C. However, no decline in concentration of S100B was observed in cerebrospinal fluid samples after 14 days when stored at 2-8°C. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and sodium azide, resulting in the final concentration of 0.03% and 0.1%, respectively.

Sample	Incubation Temp, Period	Cerebrospinal fluid (pg/ml)
1	-20°C	148.3
	2-8°C, 1 day	142.9
	2-8°C, 7 days	141.7
	2-8°C, 14 days	149.2
2	-20°C	190.3
	2-8°C, 1 day	202.5
	2-8°C, 7 days	196.4
	2-8°C, 14 days	188.2
3	-20°C	183.1
	2-8°C, 1 day	183.1
	2-8°C, 7 days	168.3
	2-8°C, 14 days	172.8

Effect of Freezing/Thawing

Concentration of human S100B in cerebrospinal fluid samples declined more than 30% after repeated (3x) freeze/thaw cycles. It is strongly recommended to avoid repeated freezing/thawing of the samples.

Sample	Number of f/t cycles	Cerebrospinal fluid (pg/ml)
1	1x	221.3
	3x	132.1
	5x	93.1
2	1x	218.8
	3x	191.5
	5x	174.3
3	1x	304.8
	3x	203.7
	5x	191.0

Comparison of serum and CSF samples

Serum samples and cerebrospinal fluids were taken from 8 patients with head injury or brain disorder and measured in the assay, results shown below:

Volunteer No.	Serum(pg/ml)	Cerebrospinal fluid (pg/ml)
1	95.7	394
2	52.3	2 194
3	31.8	485
4	42.6	479
5	0	237
6	0	890
7	0	575
8	0	893

Reference range

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 S100B levels with the assay.

14. DEFINITION OF THE STANDARD

A S100B (ββ homodimer) protein purified from human brain tissue is used as the standard in this assay. S100B is a 21 kDa protein.

15. METHOD COMPARISON

The Biovendor Human S100B ELISA has not been compared to other commercial immunoassays.

16. 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 explanations:
- Improper or inadequate washing
- Improper mixing Standards, Quality Controls or samples

17. REFERENCES

References to S100B:

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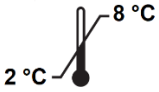
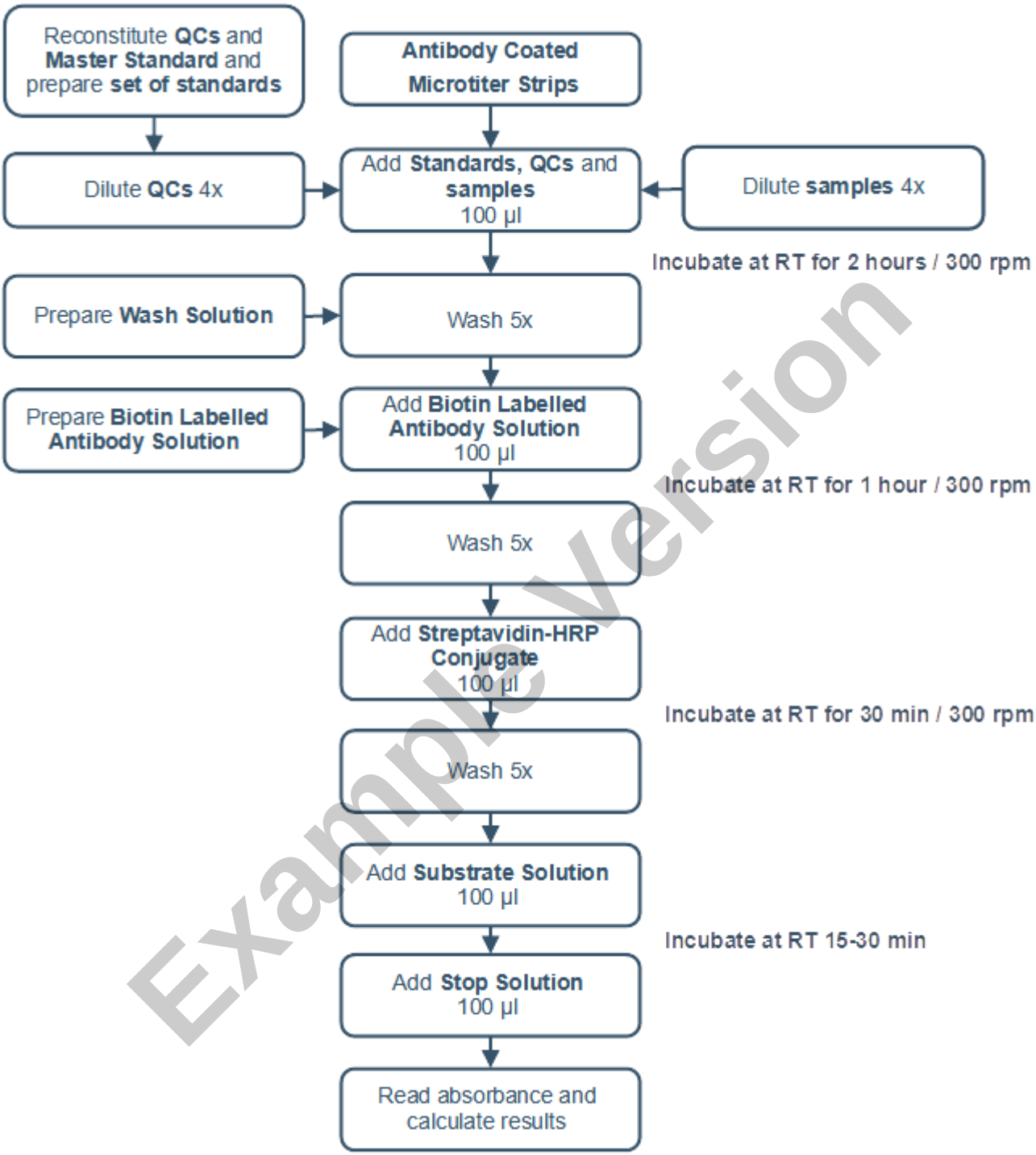
- Celikbilek A et al.: The Serum S100B Level as a Biomarker of Enteroglial Activation in Patients with Ulcerative Colitis. Int J Inflam. 2014:986525 (2014)
- Brouns R et al.: Neurobiochemical markers of brain damage in cerebrospinal fluid of acute ischemic stroke patients. Clin Chem. 56(3):451-8 (2010)

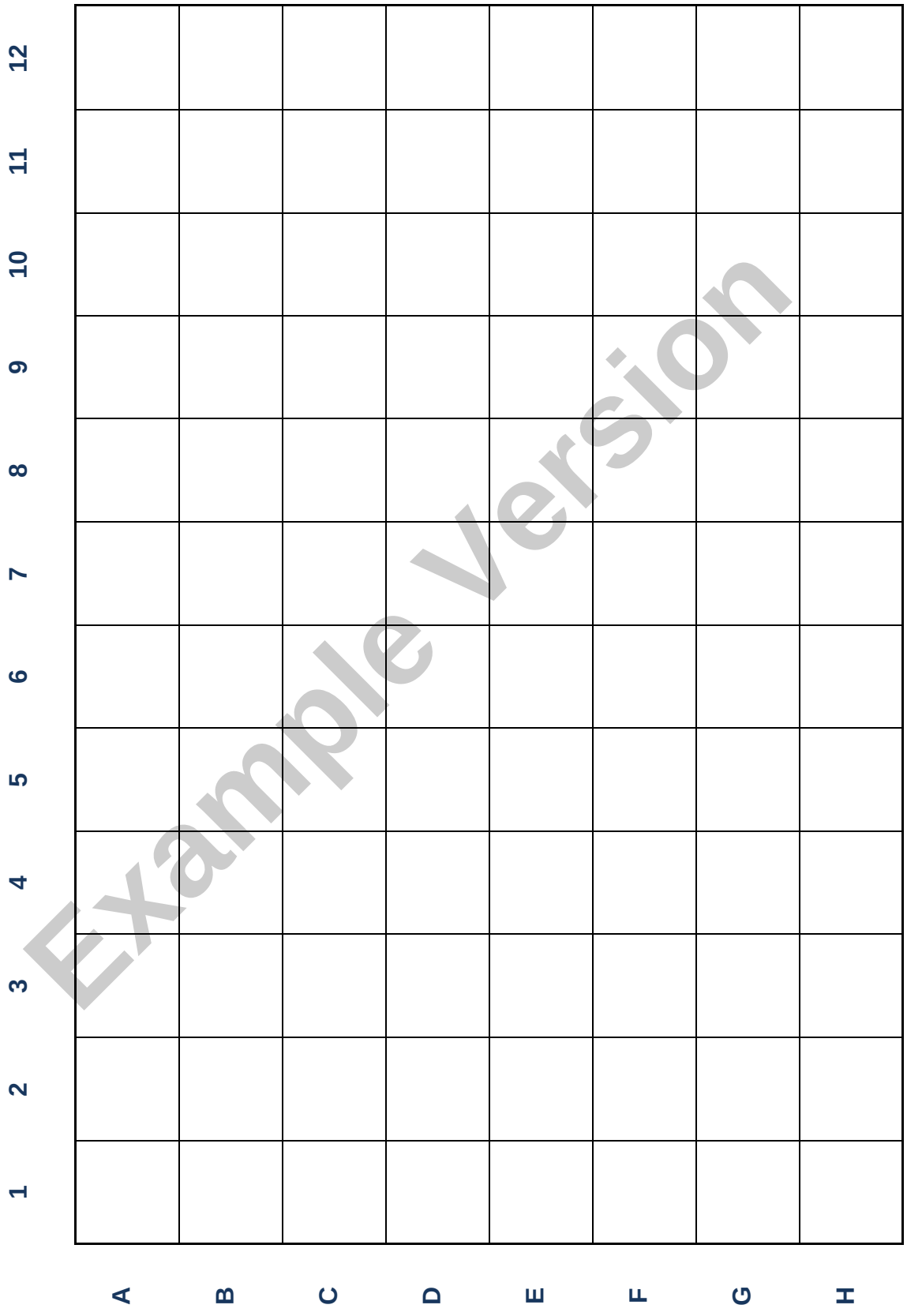
For more references on this product see our web pages at www.biovendor.com.

18. EXPLANATION OF SYMBOLS

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

19. ASSAY PROCEDURE - SUMMARY





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