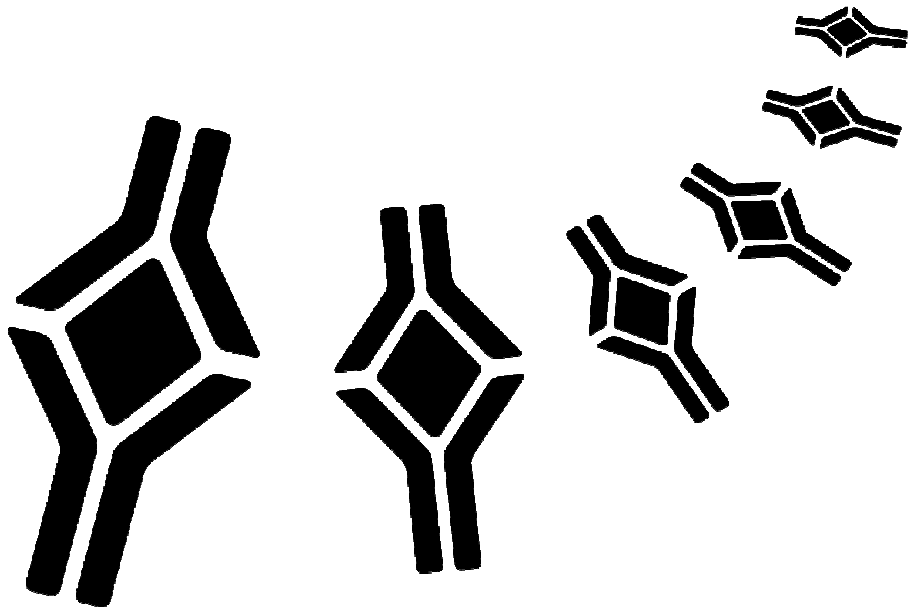
BioVendor
Research
and Diagnostic Products



HUMAN S100B ELISA

Product Data Sheet

Cat. No.: RD192090100R

For Research Use Only

VERSION 06 180810 20

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 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 5 hours
- The kit measures S100B protein in serum, heparin plasma and cerebrospinal fluid
- Assay format is 96 wells
- Quality Controls are human serum based. Animal serum is used for Master Standard and for Dilution Buffer preparation
- 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

4. TEST PRINCIPLE

In the BioVendor Human S100B ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-cow 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	0.15 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	1 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
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 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**

» **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 reconstution 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 **4000 pg/ml**.
Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	4000 pg/ml
300 µl of stock	300 µl	2000 pg/ml
300 µl of 2000 pg/ml	300 µl	1000 pg/ml
300 µl of 1000 pg/ml	300 µl	500 pg/ml
200 µl of 500 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

Dilute each concentration of standard 4x with Dilution Buffer prior to the assay, e.g. 40 µl of standard + 120 µl of Dilution Buffer for singlets, or preferably 60 µl of standard + 180 µl of Dilution Buffer for duplicates. **Mix well** (not to foam).

Stability and storage:

Standard stock solutions (4000 - 50 pg/ml) should be aliquoted and frozen at –20°C for 3 months. Avoid repeated freeze/thaw cycles.

Do not store the 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.

Biotin Labelled Antibody Conc. (100x)

Prepare the working Biotin Labelled Antibody solution by adding 1 part of Biotin Labelled Antibody Concentrate (100x) to 99 parts of Biotin-Ab Diluent.

Example (for 1 strip, i.e. 8 wells): 10 µl of Biotin Labelled Antibody Concentrate (100x) + 990 µl Biotin-Ab Diluent.

Stability and storage:

Opened Biotin Labelled Antibody Concentrate (100x) is stable 3 months when stored at 2-8°C.

Do not store diluted Biotin Labelled Antibody working 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 S100B in serum, heparin plasma and cerebrospinal fluid samples.

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.

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 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 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.

11. ASSAY PROCEDURE

1. Pipet **100 µl** of diluted Standards, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells.
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 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 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 minutes** at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C. Do not shake the plate during the incubation.
12. Stop the colour development by adding **100 µl** of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 - 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm. **The absorbance should be read within 5 minutes following step 12.**

Note 1: If some samples and standard/s have 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: 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 2000**	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 1000**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 500**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 200**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 100**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 50**	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.

Samples, Quality Controls and Standards are all diluted 4x prior to analysis, so there is no need to take this dilution factor into account.

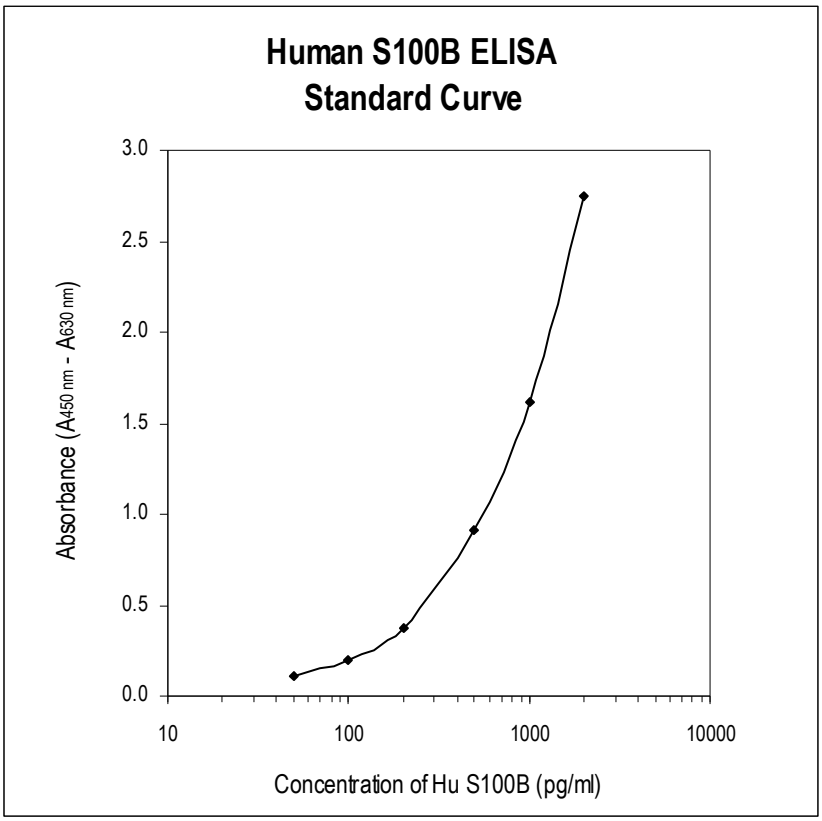


Figure 2: Typical Standard curve for Human S100B ELISA.

13. PERFORMANCE CHARACTERISTICS

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

- **Sensitivity**

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 S100B values in wells and is 15 pg/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding S100B level of 2000 pg/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the S100B concentration.

- **Specificity**

The antibodies used in this ELISA are specific for human S100B with no detectable crossreactivities to human S100A1, S100P, S100Z and neuroglobin. Approximately 2% crossreactivity to human GFAP was observed.

S100B was detected in diluted (1:1500) mouse brain homogenate samples using this ELISA.

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	yes
Cat	yes
Dog	no
Goat	yes
Hamster	yes
Horse	no
Monkey	no
Mouse	no
Pig	yes
Rabbit	yes
Rat	yes
Sheep	no

- **Precision**

Intra-assay (Within-Run) (n=6)

Sample	*Mean (pg/ml)*	*SD (pg/ml)*	*CV (%)*
1	574.7	15.7	2.7
2	1005.2	38.7	3.8

Inter-assay (Run-to-Run) (n=3)

Sample	*Mean (pg/ml)*	*SD (pg/ml)*	*CV (%)*
1	527.2	53.0	10.1
2	939.9	49.3	5.2

- **Spiking Recovery**

Serum samples were spiked with different amounts of human S100B antigen and assayed.

Sample	***O**bserved (pg/ml)*	***E**xpected (pg/ml)*	*Recovery **O**/**E** (%)*
1	150.8	-	-
	189.1	190.8	99.1
	248.2	230.8	107.5
	299.4	310.8	96.3
2	428.9	-	-
	446.7	468.9	95.3
	508.9	508.9	100.0
	574.6	588.9	97.6

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (pg/ml)*	***E**xpected (pg/ml)*	*Recovery **O**/**E** (%)*
1	-	483.7	-	-
	2x	244.1	241.8	101.0
	4x	130.8	120.9	108.2
	8x	55.8	60.5	92.2
2	-	869.3	-	-
	2x	436.5	434.7	100.4
	4x	226.2	217.3	104.1
	8x	96.0	108.7	88.3

- **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 0.1%, 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	2194
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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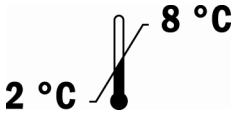
- Foerch C et al.: Elevated serum S100B levels indicate a higher risk of hemorrhagic transformation after thrombolytic therapy in acute stroke. *Stroke*. **38**(9):2491-5 (2007)

›› References to this product:

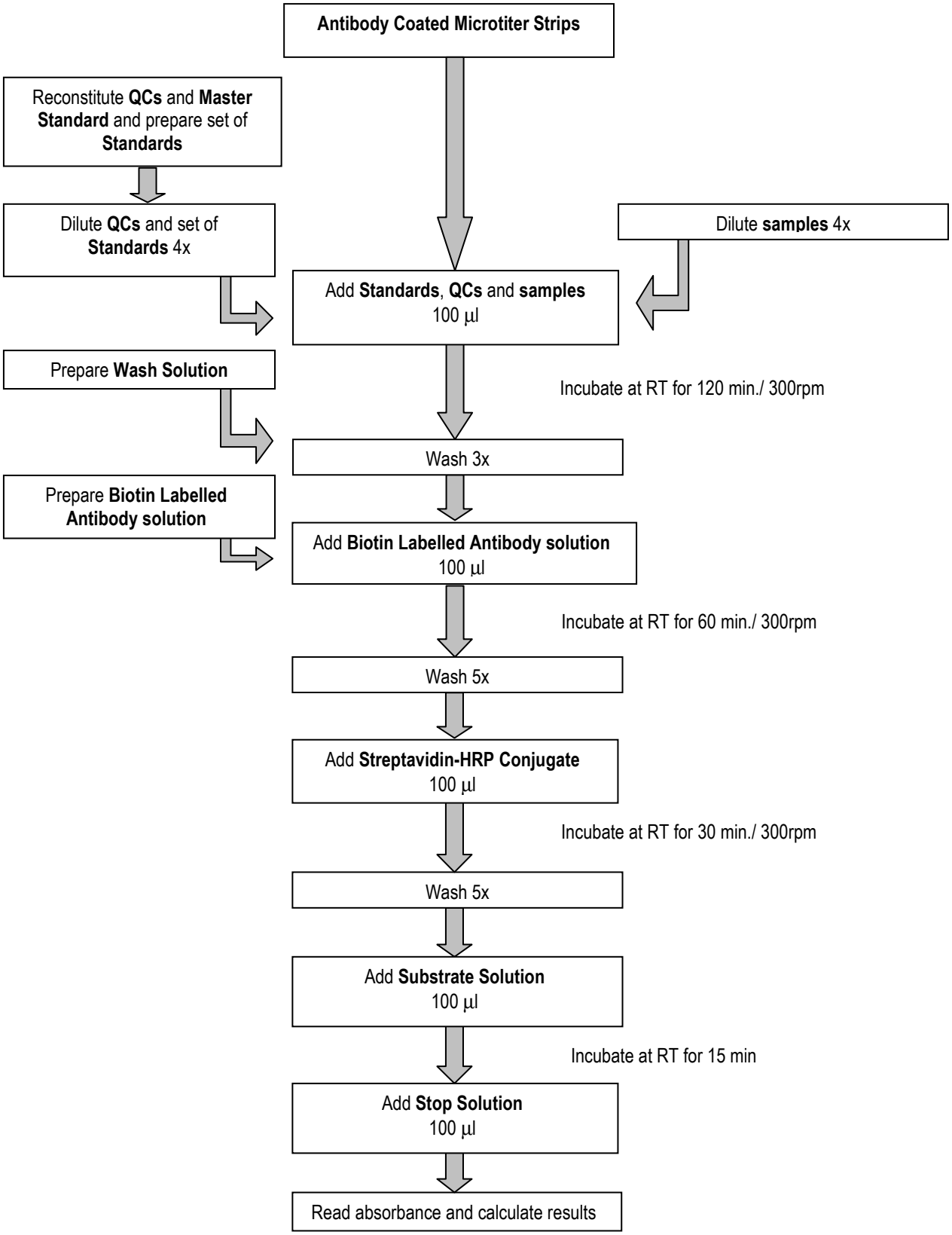
- Friel LA et al.: The calcium binding protein, S100B, is increased in the amniotic fluid of women with intra-amniotic infection/inflammation and preterm labor with intact or ruptured membranes. *J Perinat Med.* **35**(5):385-93 (2007)
- Hamed SA et al.: Oxidative stress and S-100B protein in children with bacterial meningitis. *BMC Neurol.* **9**:51 (2009)
- Misu T et al.: Marked increase in cerebrospinal fluid glial fibrillar acidic protein in neuromyelitis optica: an astrocytic damage marker. *J Neurol Neurosurg Psychiatry* **80**(5): 575-7 (2009)

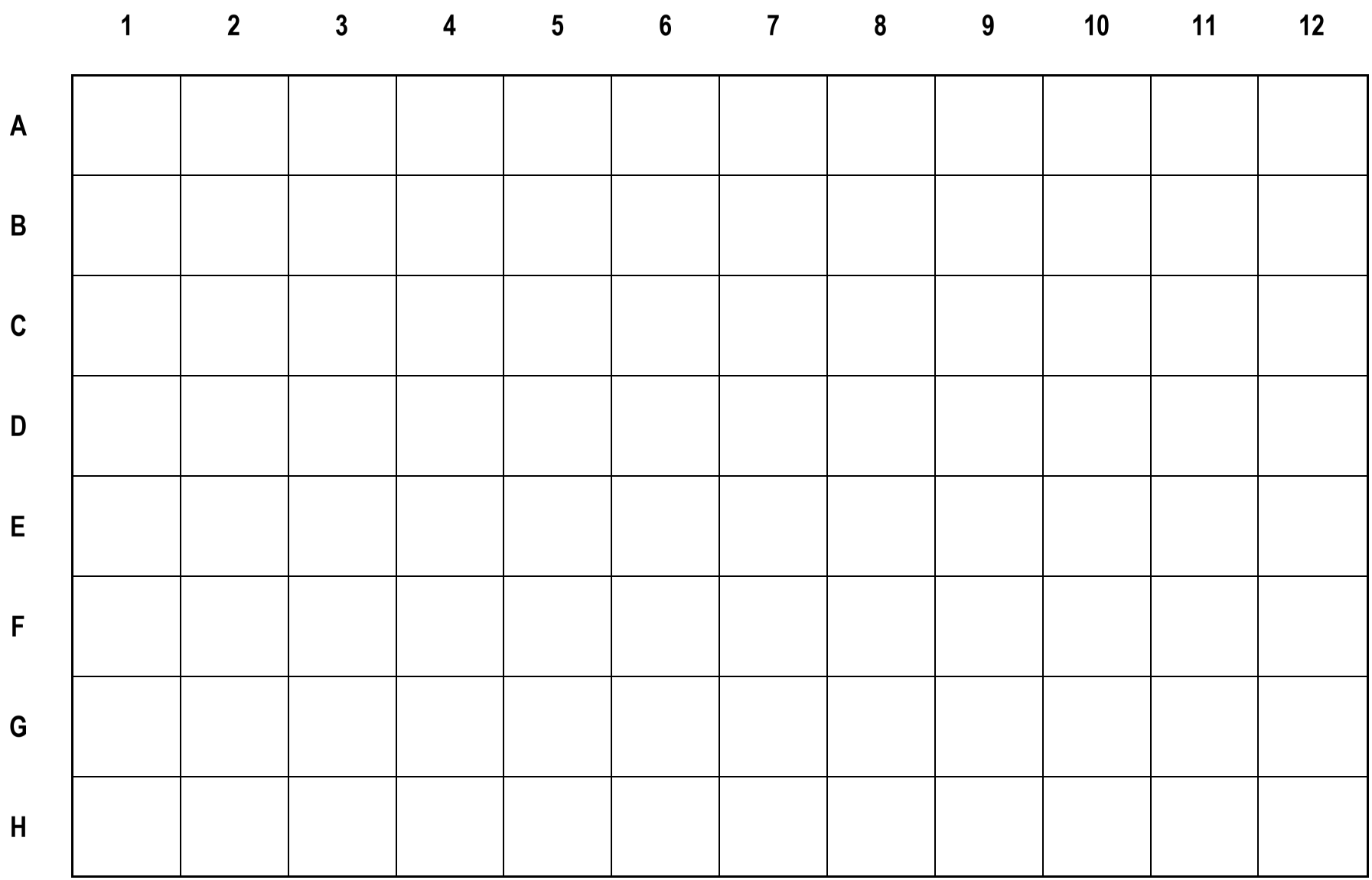
›› For more references on this product see our WebPages at www.biovendor.com

18. 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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NOTES

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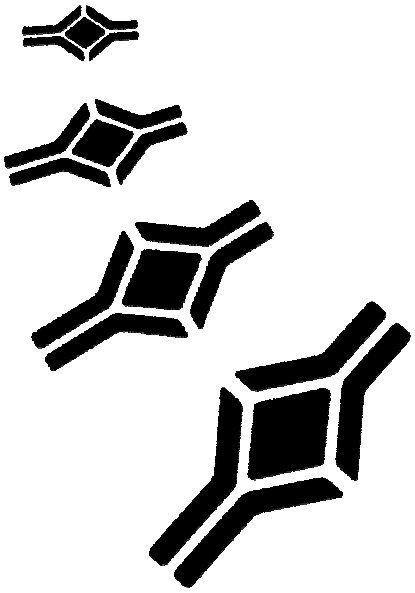

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