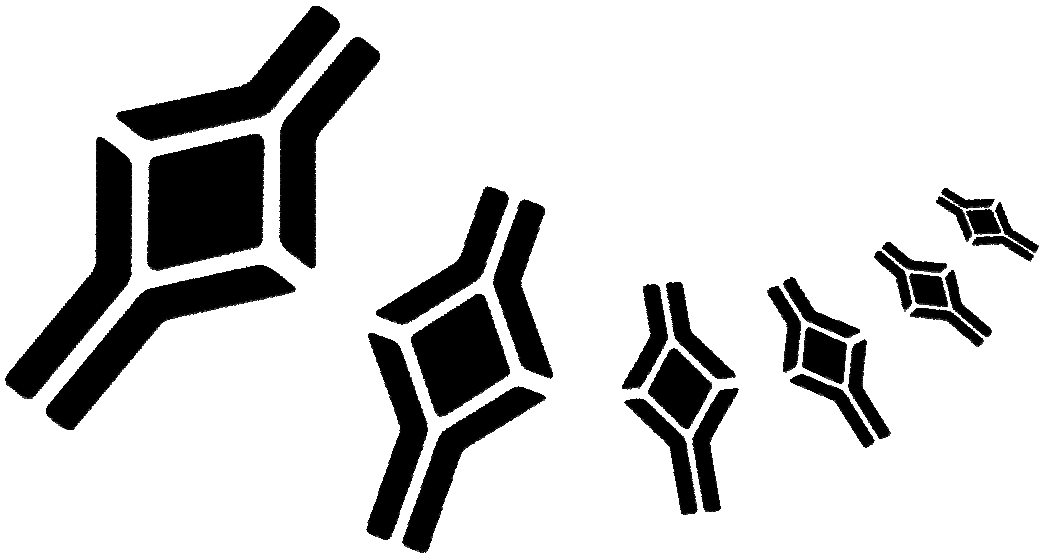
BioVendor
Research
and Diagnostic Products



HUMAN ARGINASE LIVER TYPE ELISA

Product Data Sheet

Cat. No.: CS058

For Research Use Only

VERSION 03 22062011

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 CS058 (the original catalog number RD193028000R) Human Arginase Liver-Type ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human Arginase I (Liver-Type).

» Features

- **It is intended for research use only.**
- The total assay time is less than 3 hours.
- The kit measures total serum Arginase I (Liver-Type).
- Assay format is 96 wells.
- Quality Controls are human serum based. No animal sera are used.
- Standard is recombinant protein based.
- Serum samples require very careful preparation. The erythrocytes have to be spinned down immediately (within few seconds) after taking blood to avoid hemolysis and contamination of the sample with erythrocyte arginase.
- Components of the kit are provided ready to use, concentrated or lyophilized.

2. STORAGE, EXPIRATION

Place the lyophilized Master Standards and Quality Controls at –20 °C after the kit delivery.
Store the other kit components at 2-8°C.

Under these conditions the kit is stable till the expiry date is over. (See the expiry date indicated on the kit label).

For stability of opened reagents see Chapter 9.

3. INTRODUCTION

Arginase [EC 3.5.3.1; L-arginine aminohydrolase] is an enzyme that hydrolyzes L-arginine to L-ornithine and urea in the urea cycle. Two forms of arginase exists which are designed as arginase I and arginase II. Liver-type arginase I is expressed primarily in the liver and to some extend in the erythrocytes. Arginase II is expressed in many extrahepatic tissues, such as brain, spinal cord, kidney, small intestine and mammary gland. Although arginase I and arginase II have similar enzyme activities, they have different pI, immunological reactivity and are encoded by different genes. Human arginase I is a 35 kDa protein circulating in blood probably as a homotrimer.
Circulating liver-type arginase was clinically used as a liver specific marker which may reflect not only early occurrence of liver injury but also early termination of liver injury. The measurement of liver-type arginase is clinically applicable for monitoring conditions of patients with liver disorders or pre- and postoperative conditions of patients who received partial hepatectomy with quicker normalization in comparison with aminotransferases (ALT and AST).
Recently, arginase I gene was found to be one of the most prominent among asthma genes. *In situ* hybridization demonstrated marked staining of arginase I in submucosal inflammatory lesions and arginase activity increased in allergen challenged lungs.
Finally, it was found that both arginase I was the most significantly up-regulated protein in the murine spinal cord during experimental autoimmune encephalomyelitis. The results indicated that arginase I played important roles in autoimmune inflammation in the central nervous system.

Areas of investigation:
Blood Pressure Regulation and NO Metabolism

4. TEST PRINCIPLE

In the BioVendor Human Arginase Liver Type ELISA Standards, Quality Controls and samples are incubated in microplate wells pre-coated with monoclonal anti-human arginase antibody. After 60 minutes incubation and washing, monoclonal anti-human arginase antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with captured arginase. Following another washing step, the remaining HRP 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 spectrophotometrically at 450 nm. The absorbance is proportional to the concentration of arginase. 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.
- 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
Conjugate Solution	ready to use	13 ml
Master Standard	lyophilized	2 vials
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
Standard Diluent	ready to use	2 ml
Dilution Buffer	ready to use	13 ml
Wash Solution Conc. (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml
Product Data Sheet + Certificate of Analysis	-	1 pc

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision pipettes to deliver 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
- 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.

Conjugate Solution
Standard 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 Arginase Master Standard
Reconstitute the lyophilized Master Standard with **150 µl** of deionized (distilled) water just prior to the assay. Let it dissolve for 15 minutes and mix thoroughly. The resulting concentration of the human arginase in the stock solution is **320 ng/ml.**

Prepare set of standards using Standard Diluent as follows:

Volume of Standard	Standard Diluent	Concentration
Stock	-	**320 ng/ml**
75 µl of stock	75 µl	160 ng/ml
75 µl of 160 ng/ml	75 µl	80 ng/ml

75 µl of 80 ng/ml	75 µl	40 ng/ml
75 µl of 40 ng/ml	75 µl	20ng/ml
75 µl of 20 ng/ml	75 µl	10 ng/ml
75 µl of 10 ng/ml	75 µl	5 ng/ml

Dilute prepared Standards 4x with Dilution Buffer prior to the assay, e.g. 60 µl of Standard + 180 µl of Dilution Buffer for duplicates.

Stability and storage:

Stability of the reconstituted Master Standard and diluted Standards is limited; they have to be prepared just before the use in ELISA (within 30 min).

Do not store the diluted Standard solutions.

Quality Controls HIGH, LOW

Reconstitute each Quality Control (HIGH and LOW) with **60 µl** of deionized (distilled) water just prior to the assay. Let it dissolve for 15 minutes and mix thoroughly.

Dilute Quality Controls 4x with Dilution Buffer prior to the assay, e.g. 60 µl of 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 month. Avoid repeated freeze/thaw cycles.

Do not store the diluted Quality Controls.

Wash Solution Conc. (10x)

Dilute Wash Solution Conc. (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 100 ml of Wash Solution Conc. (10x)+ 900 ml of distilled water for use of all 96-wells.

Stability and storage:

The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Conc. (10x) is stable 3 months when stored at 2-8°C.

10. PREPARATION OF SAMPLES

The kit measures human Arginase I (Liver-Type) in serum and cerebrospinal fluid (CSF).

Stability and storage:
Samples should be assayed immediately after collection or should be stored at -20°C, or preferably at -70°C (then the stability is at least 1 year). 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. Do not store the diluted samples.

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- **Sera:**

It is recommended to ***spin the erythrocytes down immediately (within few seconds) after taking blood***. It is not possible to get reliable results when measuring arginase in normal serum. Trace hemolysis and contamination of serum with erythrocyte arginase causes false increased results

Dilute serum samples just prior to the assay 4x with Dilution Buffer, e.g. 60 µl of sample + 180 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

- **CSF:**

Dilute CSF samples just prior to the assay 2x with Dilution Buffer, e.g. 120 µl of sample + 120 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

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 **1 hour**, 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 Conjugate 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 **3-times** with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add **100 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
8. Incubate the plate for **10 minutes** at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C. Do not shake the plate during the incubation.
9. Stop the colour development by adding **100 µl** of Stop Solution.
10. Determine the absorbance by reading the plate at 450 nm. (Optionally, to measure in dual wavelength mode 620-650 nm filter can be used to measure the reference absorbance. The absorbance should be read within 5 minutes following step 9).

Note: If the microplate reader is not capable of reading absorbance greater than the absorbance of the highest standard, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine arginase concentration of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	Standard 320	QC HIGH	Sample 7	Sample 15	Sample 23	Sample 31
B	Standard 160	QC LOW	Sample 8	Sample 16	Sample 24	Sample 32
C	Standard 80	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
D	Standard 40	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
E	Standard 20	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
F	Standard 10	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
G	Standard 5	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
H	Blank	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38

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 at 450 nm (Y) of Standards against log of the known concentration (X) of Standards, using the four-parameter algorithm. Results are reported as concentration of human Arginase I (Liver-Type) ng/ml in samples.

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

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

The measured concentration of cerebrospinal fluid samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted 2x prior to the assay, while standards and Quality Controls are diluted 4x (e.g. 8.75 ng/ml (from standard curve) x 0,5 (dilution factor) = 4.375 ng/ml).

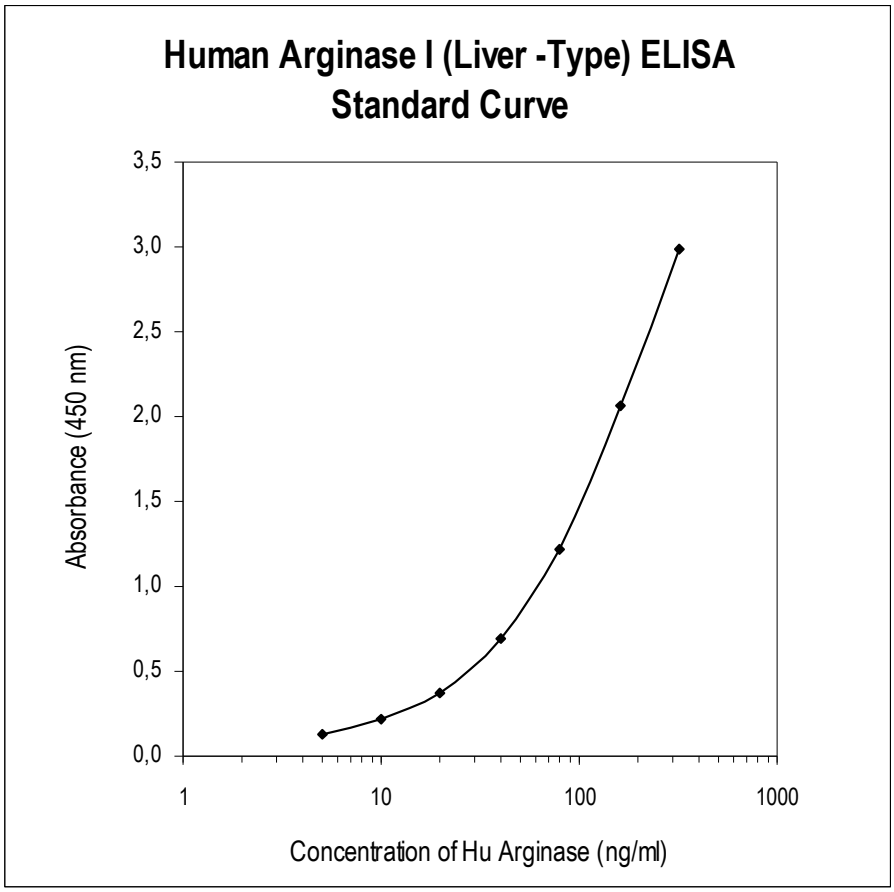

Figure 2: Typical Standard Curve for Human Arginase Liver Type ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Arginase Liver Type ELISA are presented in this chapter.

- **Sensitivity**

Limit of Detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A_{blank} + $3xSD_{blank}$) is calculated from the real human Arginase I (Liver-Type) values in wells and is 0.5 ng/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding Arginase I (Liver-Type) level of 320 ng/ml should be repeated with more diluted samples (e.g. 8x). Dilution factor needs to be taken into consideration in calculating the Arginase I (Liver-Type) concentration.

- **Specificity**

Since Arginase I (Liver-Type) exists also in erythrocytes, the erythrocyte-derived arginase cross-reacts and hemolytic sera cannot be used in this assay. Low concentrations of the erythrocyte-derived arginase in apparently non-hemolytic sera can be subtracted. The erythrocytes have to be spinned down immediately (within few seconds) after taking blood to avoid hemolysis and contamination of the sample with erythrocyte Arginase.

The antibodies used in this ELISA are specific for natural and recombinant human Arginase I (Liver-Type). No cross-reactivity has been observed for human Arginase II.

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

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	8.75	0.59	6.7
2	28.74	1.41	4.9

Inter assay (Run-to-Run) (n=8)

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	8.83	0.76	8.6
2	29.65	2.16	7.3

- **Spiking Recovery**

Serum samples were spiked with different amounts of human Arginase I (Liver-Type) and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	8.69	-	-
	24.96	28.69	87
	40.89	48.69	84
	81.59	88.69	92
2	27.93	-	-
	42.66	47.93	89
	56.38	67.93	83
	90.66	107.93	84

- **Linearity**

Serum samples (4x diluted) were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	-	45.27	-	-
	2x	19.78	22.64	87
	4x	11.92	11.32	105
	8x	4.78	5.66	85
2	-	81.77	-	-
	2x	37.65	40.89	92
	4x	18.22	20.44	89
	8x	9.02	10.22	88

- **Reference ranges**

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 Arginase I (Liver-Type) levels with the assay.

14. DEFINITION OF THE STANDARD

The Standard used in this kit is a recombinant protein expressed in E.coli.

15. METHOD COMPARISON

BioVendor Human Arginase Liver Type ELISA was not compared to the 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 explanation:

- Improper or inadequate washing
- Improper mixing Standards, Quality Controls or samples

17. REFERENCES

References to Human Arginase Liver Type ELISA:

- Ikemoto M, Tsunekawa S, Awane M, Fukuda Y, Murayama H, Igarashi M, Ngata A, Kasai Y, Totani M: A useful ELISA system for human liver-type arginase, and its utility in diagnosis of liver diseases. *Clin Biochem*. **34**, 455-461 (2001).
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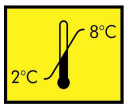
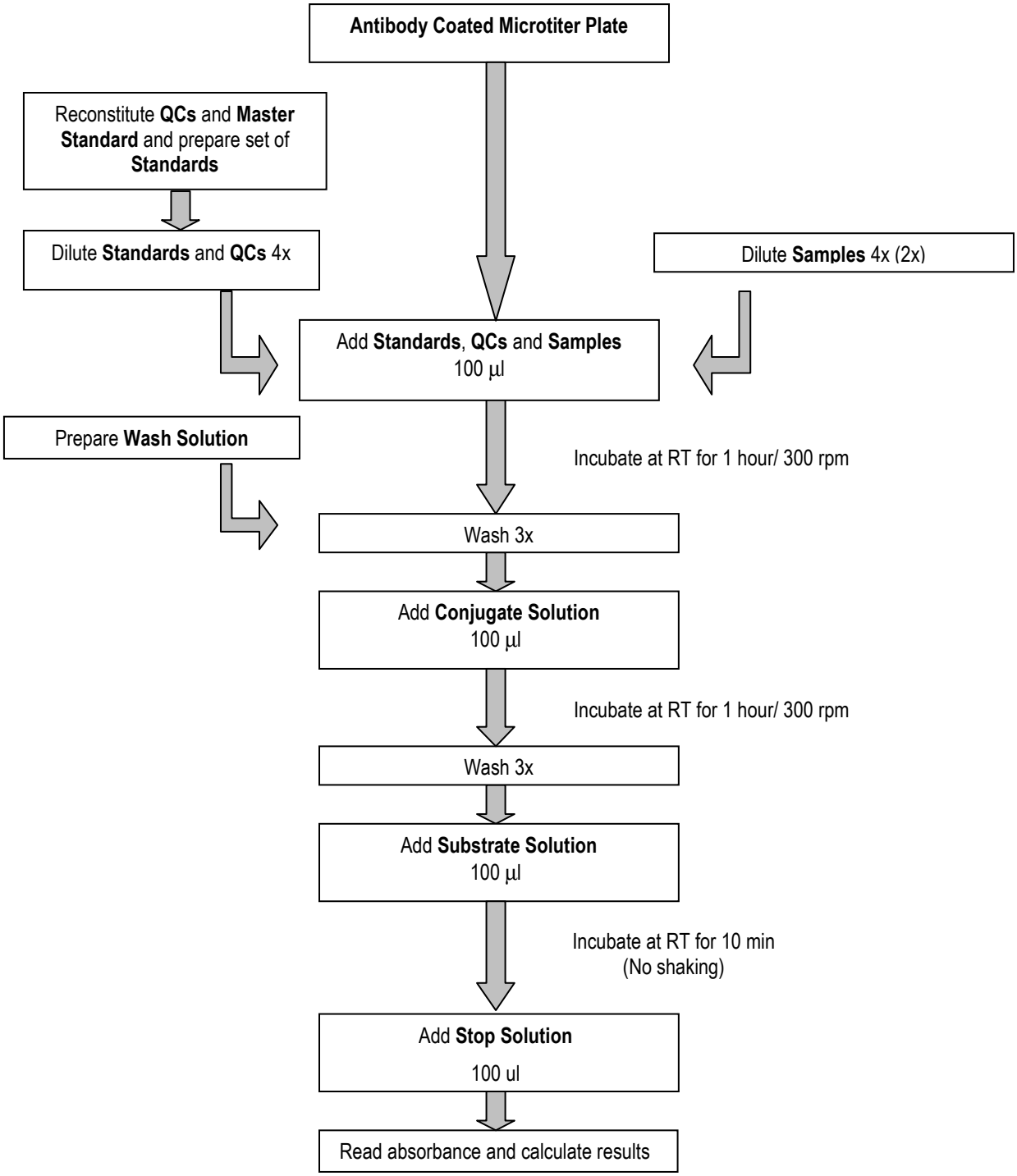
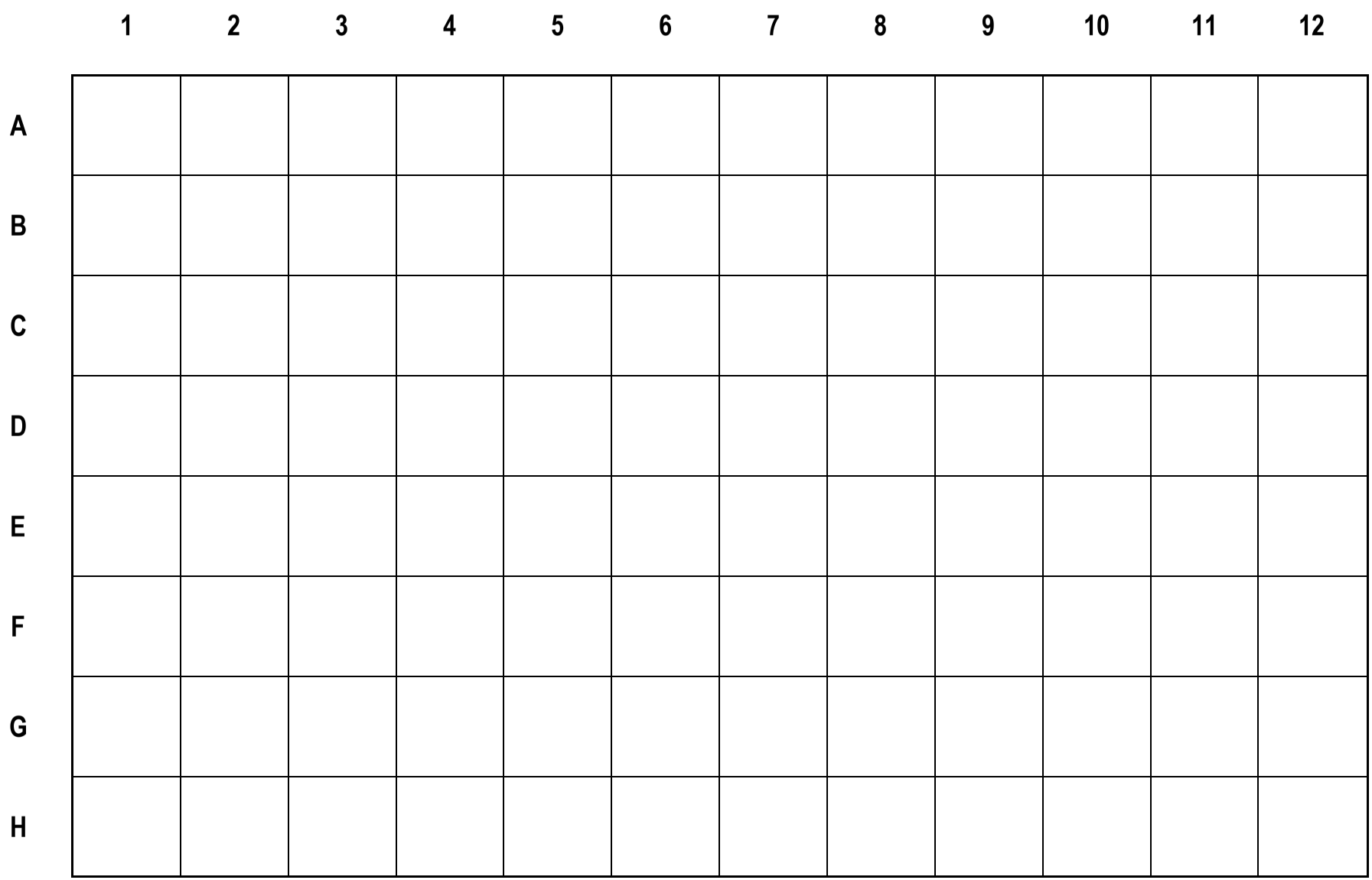
For more references on this product see our WebPages at www.biovendor.com

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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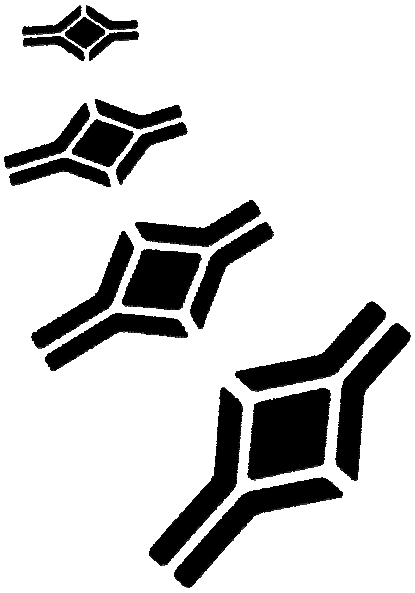

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