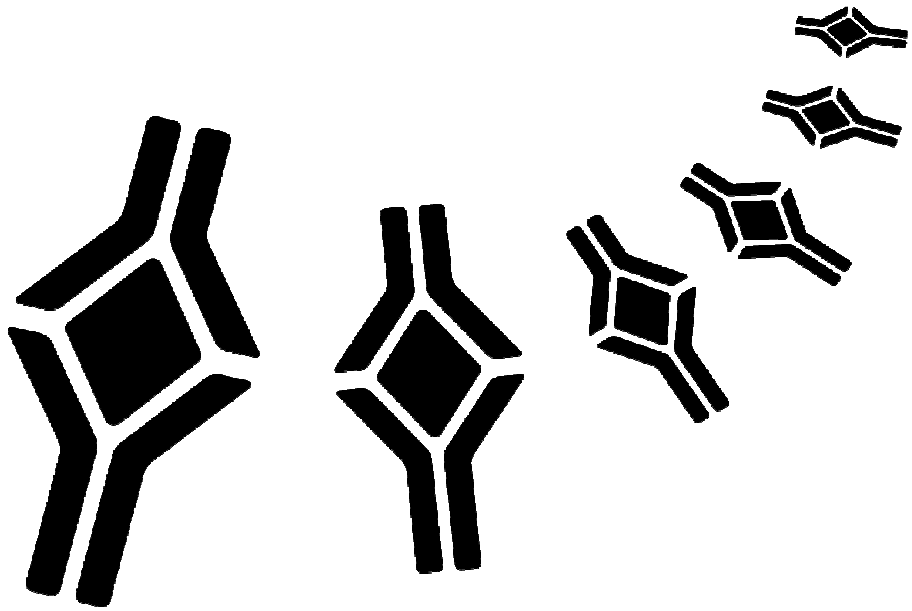
BioVendor
Research
and Diagnostic Products



# RAT CLUSTERIN ELISA

Product Data Sheet

Cat. No.: RD391034200R

For Research Use Only



VERSION 98 130510 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 RD391034200R Rat Clusterin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of rat clusterin.

### Features

- **It is intended for research use only**
- The total assay time is less than 3.5 hours
- The kit measures clusterin in rat serum and rat urine
- Assay format is 96 wells
- Quality Controls are rat serum based. No human sera are used
- Standard is recombinant 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

Clusterin (Apolippoprotein J; SP-40,40; TRPM-2; SGP-2; pADHC-9; CLJ; T64; GP III; XIP8) is a highly conserved disulfide-linked secreted heterodimeric glycoprotein of 75-80 kDa but truncated forms targeted to nucleus have also been identified.
Clusterin is highly conserved across species, showing 70-80% identity at the amino acid level amongst mammals, and numerous variants and isoforms have been describe. The protein is constitutively secreted by a number of cell types including epithelial and neuronal cells and is a major protein in physiological fluids including plasma, milk, urine, cerebrospinal fluid and semen. Due to its wide tissue distribution many diverse physiological functions have been attributed to clusterin including sperm maturation, membrane recycling, lipid transportation, tissue remodelling, complement inhibition and cell-cell or cell-substratum interactions. Moreover, it was propose, that clusterin functions is as an extra cellular chaperon that stabilizes stressed proteins in a folding-competent state and protein has also been implicated in programmed cell death. Another defining prominent of clusterin is its induction in many severe physiological disturbances states including kidney degenerative diseases, prostate and vesicle carcinogenesis, ovarian cancer and several neurodegenerative conditions.
Recent study demonstrate, that serum clusterin level in human increases significantly in diabetic type II patients and in patients with developing coronary heart disease, or myocardial infarction. These date raise the possibility that elevated clusterin levels in serum may represent a strong indication of vascular damage.
Interesting study determine that urinary clusterin levels in the rat correlate with severity of tubular damage and may help to differentiate between glomerular and tubular injuries.

<u>Areas of investigation:</u>
Coronary heart diseases
Neurodegenerative diseases
Kidney degenerative disease
Renal tubular damage

# 4. TEST PRINCIPLE

In the BioVendor Rat Clusterin ELISA, Standards, Quality Controls and samples are incubated in microplate wells pre-coated with polyclonal anti-rat clustein antibody. After 60 minutes incubation and washing, biotin labelled polyclonal anti-rat clusterin antibody is added and incubated with captured clusterin for 60 minutes. 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 clusterin. 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 Conc. (50x) | concentrated | 0.26 ml |
| Streptavidin-HRP Conjugate | ready to use | 13 ml |
| Master Standard | lyophilized | 2 vials |
| Quality Control HIGH | lyophilized | 2 vials |
| Quality Control LOW | lyophilized | 2 vials |
| Biotin-Ab Diluent | ready to use | 13 ml |
| Dilution Buffer | ready to use | 50 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 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 550-650nm)
- 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 month 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 month when stored at 2-8°C.

- **Assay reagents supplied concentrated or lyophilized:**

**Rat Clusterin Master Standard**
**Refer to the Cetrificate 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 clusterin in the stock solution is **128 ng/ml**.

Prepare set of standards (128 ng/ml - 4 ng/ml for measurement in serum samples or 128 ng/ml - 2 ng/ml for measurement in urine samples) using Dilution Buffer as follows:

| *Volume of Standard* | *Dilution Buffer* | *Concentration* |
|---|---|---|
| Stock | - | 128 ng/ml |
| 300 µl of stock | 300 µl | 64 ng/ml |
| 300 µl of 64 ng/ml | 300 µl | 32 ng/ml |
| 300 µl of 32 ng/ml | 300 µl | 16 ng/ml |
| 300 µl of 16 ng/ml | 300 µl | 8 ng/ml |
| 300 µl of 8 ng/ml | 300 µl | 4 ng/ml |
| 300 µl of 4 ng/ml | 300 µl | 2 ng/ml |

**Prepared Standards are ready to use, do not dilute them.**
Stability and storage:
**Do not store the Standard stock solution and set of standards.**

**Quality Controls HIGH, LOW**
**Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Control concentration!!!**
Reconstitute each Quality Control (HIGH and LOW) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).
**Reconstituted Quality Controls are ready to use, do not dilute them.**
Stability and storage:
**Do not store the reconstituted Quality Controls.**

**Biotin Labelled Antibody Conc. (50x)**
Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (50x) with 49 parts Biotin-Ab Diluent. Example: 20 µl of Biotin Labelled Antibody Concentrate (50x) + 980 µl of Biotin-Ab Diluent for 1 strip (8 wells).
Stability and storage:
Opened Biotin Labelled Antibody Concentrate (50x) is stable 3 months when stored at 2-8°C.
**Do not store the diluted Biotin Labelled Antibody 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 rat clusterin in serum and urine.

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 samples:**
Dilute samples just prior to the assay 2 000x with Dilution Buffer, in two steps as follows:
**Dilution A** (40x):
Add 5 µl of sample into 195 µl of Dilution Buffer and **mix well** (not to foam). Vortex is recommended.
**Dilution B** (50x):
Add 5 µl of Dilution A into 245 µl of Dilution Buffer to prepare final dilution (2000x) and **mix well** (not to foam). Vortex is recommended.
Stability and storage:
Serum 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.**

**Urine samples:**
Dilute urine samples just prior to the assay 10x with Dilution Buffer, e.g. 15 µl of sample + 135 µl of Dilution Buffer for singlets or 25 µl of sample + 225 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.
Stability and storage:
Urine samples should be stored at -70°, under this conditions samples are stable minimally 3 months. Avoid repeated freeze/ thaw cycles.
**Do not store the diluted samples.**

*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. 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 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 **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 Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for **30 min**, shaking at ca. 300 rpm on an orbital microplate shaker.
9. 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.
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** for measurement in serum samples or **20 minutes** for measurement in urine samples at room temperature. The incubation time may be extended [up to 20 or 30 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: 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 rat clusterin 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 128** | **Blank** | Sample 8 | Sample 16 | Sample 24 | Sample 32 |
| **B** | **Standard 64** | Sample 1 | Sample 9 | Sample 17 | Sample 25 | Sample 33 |
| **C** | **Standard 32** | Sample 2 | Sample 10 | Sample 18 | Sample 26 | Sample 34 |
| **D** | **Standard 16** | Sample 3 | Sample 11 | Sample 19 | Sample 27 | Sample 35 |
| **E** | **Standard 8** | Sample 4 | Sample 12 | Sample 20 | Sample 28 | Sample 36 |
| **F** | **Standard 4** | 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 rat clusterin 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.

**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. 20 ng/ml (from standard curve) x 2000 (dilution factor) = 40 000 ng/ml = 40 µg/ml.**

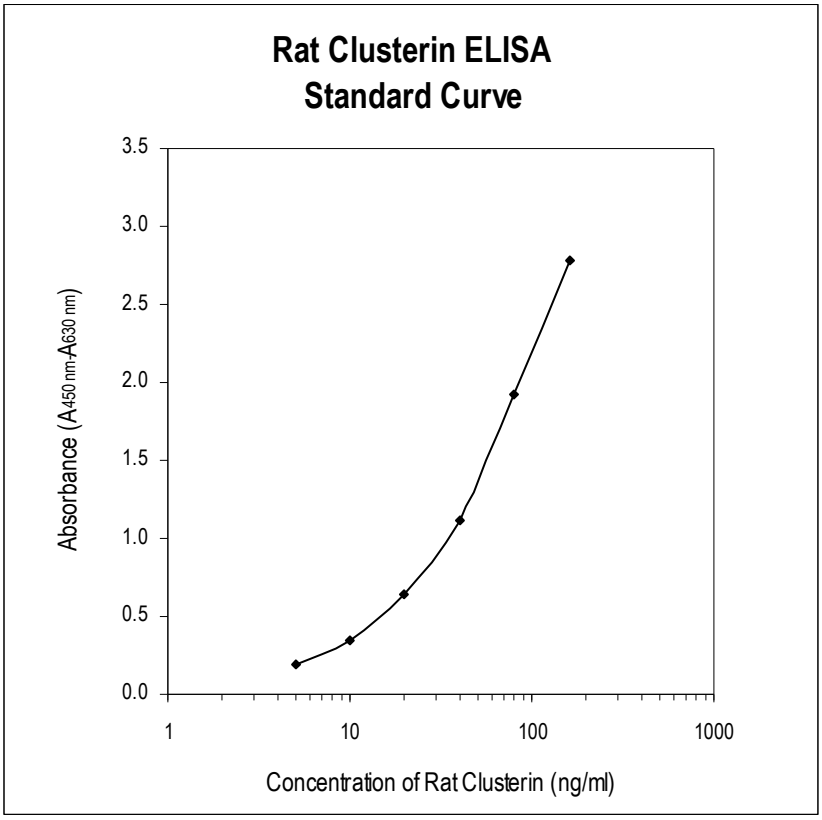



*Figure 2: Typical Standard Curve for Rat Clusterin ELISA.*

# 13. PERFORMANCE CHARACTERISTICS

**Typical analytical data of BioVendor Rat Clusterin 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 rat clusterin values in wells and is 0.7 ng/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding clusterin level of 128 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the clusterin concentration.

- **Specificity**

The antibodies used in this ELISA are specific for rat clusterin.

Human serum and 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 |
| Human | no |
| Monkey | no |
| Mouse | yes |
| Pig | no |
| Rabbit | no |
| Sheep | no |

» **Presented results are multiplied by respective dilution factor**

- **Precision**

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

| *Sample* | *Mean (µg/ml)* | *SD (µg/ml)* | *CV (%)* |
|---|---|---|---|
| 1 | 31.6 | 1.23 | 3.9 |
| 2 | 37.6 | 1.81 | 4.8 |

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

| *Sample* | *Mean (µg/ml)* | *SD (µg/ml)* | *CV (%)* |
|---|---|---|---|
| 1 | 29.3 | 1.61 | 5.5 |
| 2 | 37.6 | 2.33 | 6.2 |

- **Spiking Recovery**

Serum samples were spiked with different amounts of rat clusterin and assayed.

| *Sample* | ***O**bserved (µg/ml)* | ***E**xpected (µg/ml)* | *Recovery **O/E** (%)* |
|---|---|---|---|
| 1 | 26.9 | - | - |
| | 130.9 | 129.3 | 101.2 |
| | 76.9 | 78.1 | 98.5 |
| | 54.0 | 52.5 | 102.9 |
| 2 | 37.2 | - | - |
| | 90.6 | 101.2 | 89.5 |
| | 65.6 | 69.2 | 94.8 |
| | 52.0 | 53.2 | 97.7 |

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

| *Sample* | *Dilution* | ***O**bserved (µg/ml)* | ***E**xpected (µg/ml)* | *Recovery **O/E** (%)* |
|---|---|---|---|---|
| 1 | - | 30.9 | - | - |
| | 2x | 15.2 | 15.5 | 98.4 |
| | 4x | 8.3 | 7.7 | 107.4 |
| | 8x | 3.7 | 3.9 | 95.8 |
| 2 | - | 41.7 | - | - |
| | 2x | 20.1 | 20.9 | 96.4 |
| | 4x | 10.5 | 10.4 | 100.7 |
| | 8x | 5.3 | 5.2 | 101.7 |

- **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 rat clusterin levels with the assay.

## 14. DEFINITION OF THE STANDARD

The recombinant rat clusterin is used as the Standard. The recombinant rat clusterin, produced in *E.coli*, is 26.5 kDa protein containing 215 amino acid residues of the rat clusterin and 25 additional amino residues. The amino acid sequence of the recombinant rat cluster is 100% homologous to the amino acid sequence 146-360 of the rat clusterin precursor.

## 15. METHOD COMPARISON

BioVendor Rat Clusterin ELISA has not been compared to any other immunoassay.

# 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 clusterin:**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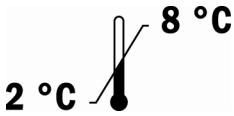
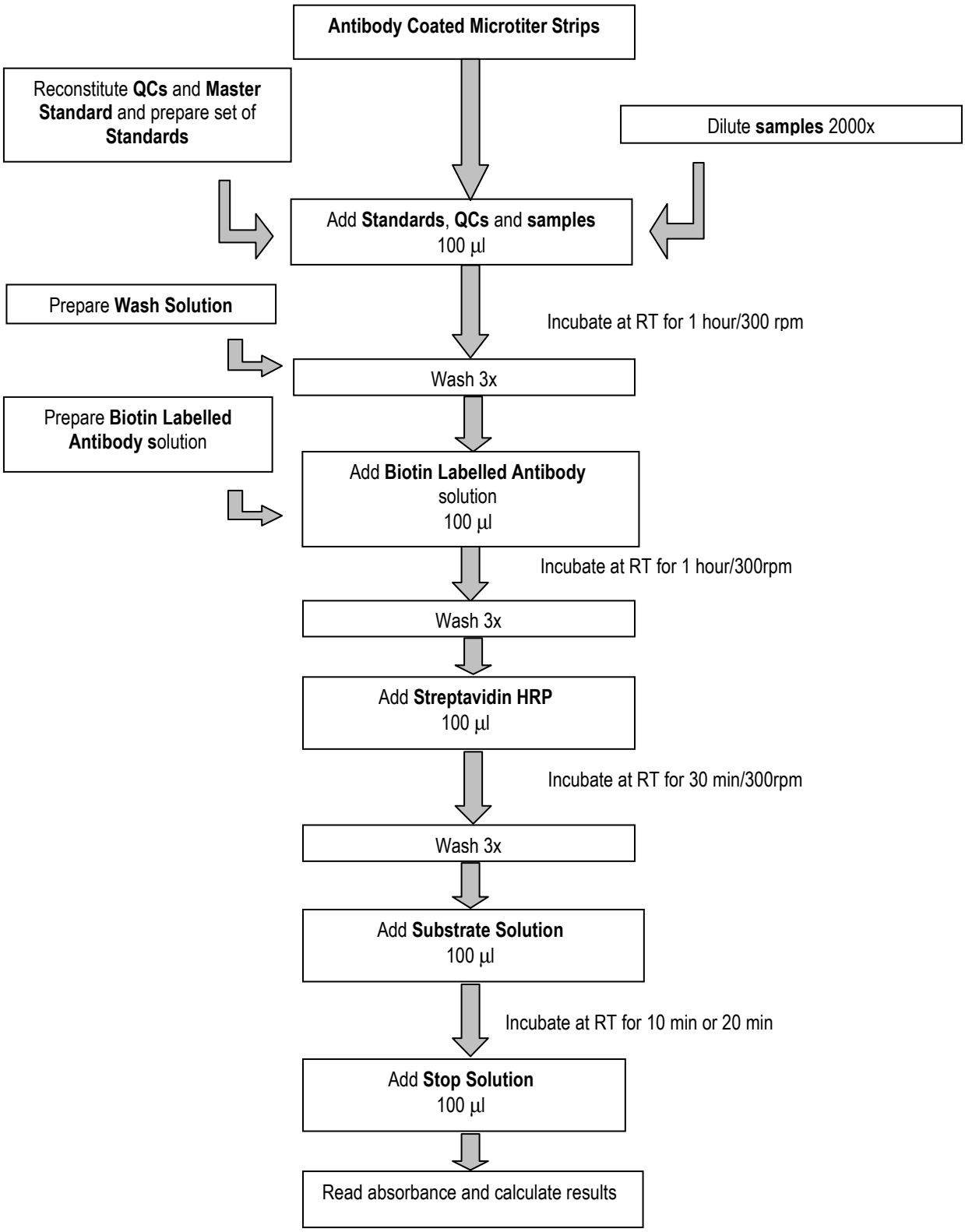
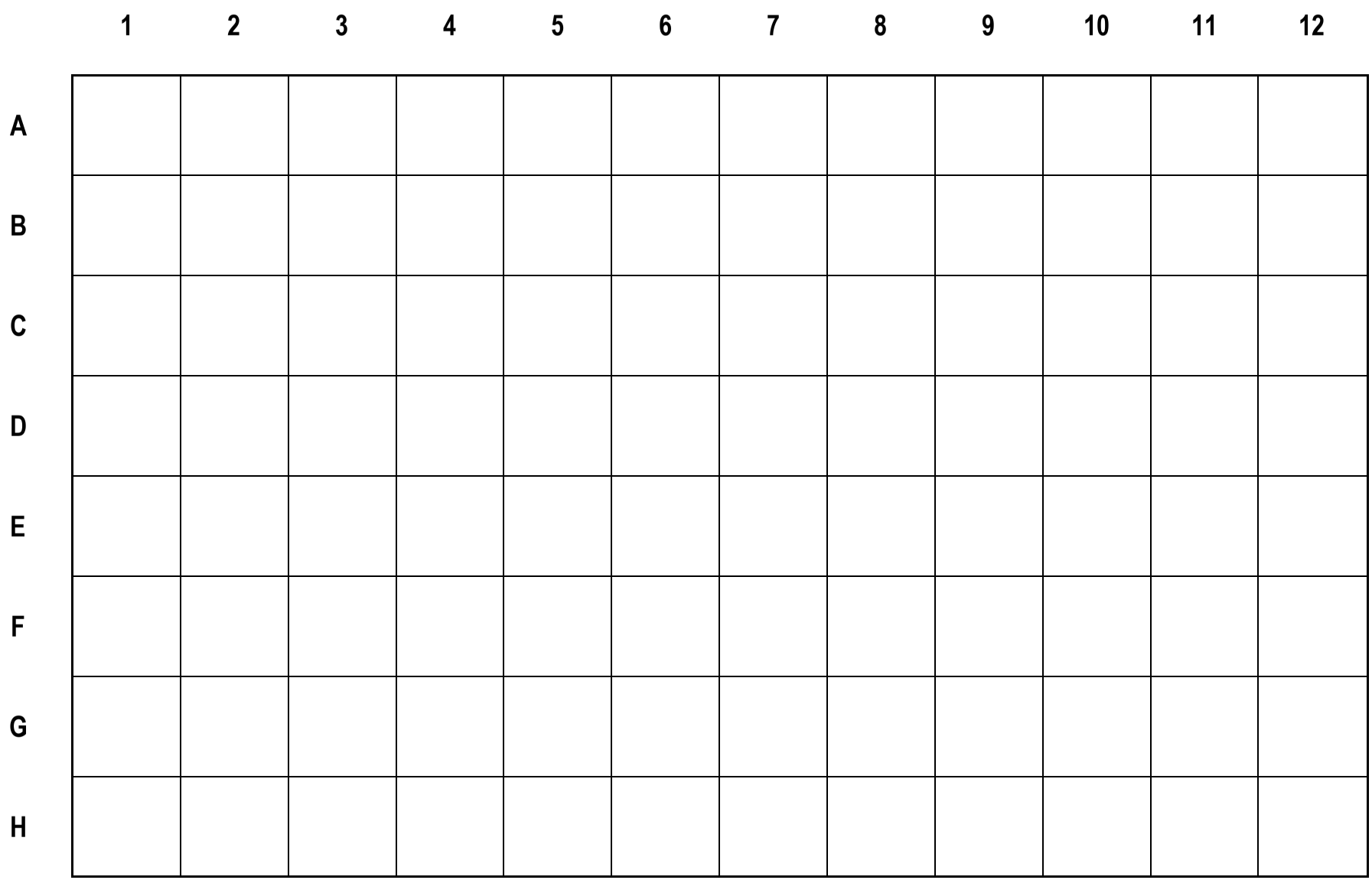
**» For more references on this product see our WebPages at www.biovendor.com**

## 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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## NOTES

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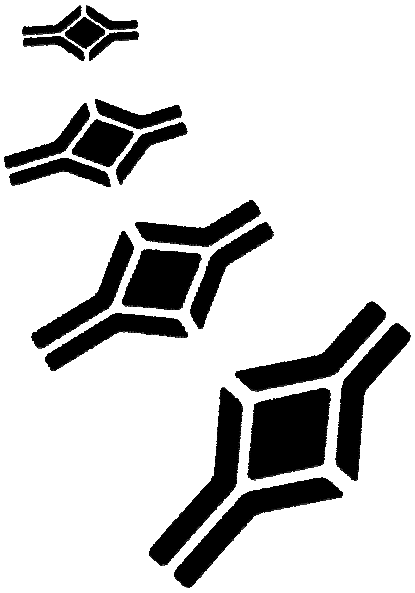


| | | | | |
|---|---|---|---|---|
| **HEADQUARTERS:**<br>**BioVendor- Laboratorní medicína a.s.** | **CTPark Modrice<br>Evropska 873** | **664 42 Modrice<br>CZECH REPUBLIC** | **Phone: +420-549-124-185<br>Fax: +420-549-211-460** | **E-mail: info@biovendor.com<br>Web: www.biovendor.com** |
| EUROPEAN UNION:<br>BioVendor GmbH | Im Neuenheimer Feld 583 | D-69120 Heidelberg<br>GERMANY | Phone: +49-6221-433-9100<br>Fax: +49-6221-433-9111 | E-mail: infoEU@biovendor.com |
| USA, CANADA AND MEXICO:<br>BioVendor LLC | 1463 Sand Hill Road<br>Suite 227 | Candler, NC 28715<br>USA | Phone: +1-828-670-7807<br>+1-800-404-7807<br>Fax: +1-828-670-7809 | E-mail: infoUSA@biovendor.com |
| CHINA - Hong Kong Office:<br>BioVendor Laboratories Ltd | Room 4008<br>Hong Kong Plaza, No.188 | Connaught Road West<br>Hong Kong, CHINA | Phone: +852-2803-0523<br>Fax: +852-2803-0525 | E-mail: infoHK@biovendor.com |
| CHINA – Mainland Office:<br>BioVendor Laboratories Ltd | Room 2405 YiYa Tower<br>TianYu Garden, No.150 | Lihe Zhong Road<br>Guang Zhou, CHINA | Phone: +86-20-87063029<br>Fax: +86-20-87063016 | E-mail: infoCN@biovendor.com |