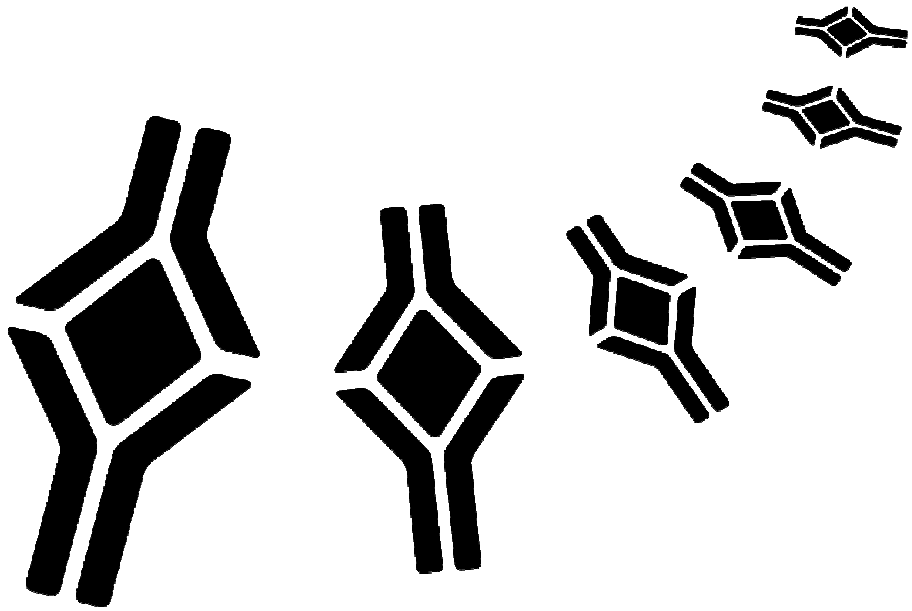
BioVendor
Research
and Diagnostic Products



# RAT UNACYLATED GHRELIN ELISA

Product Data Sheet

Cat. No.: RD394063400R

For Research Use Only



VERSION 18 240709 51

# 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 RD394063400R Rat Unacylated Ghrelin ELISA is a sandwich enzyme immunoassay for quantitative measurement of rat unacylated ghrelin in EDTA plasma and buffer solution.

### Features

- **It is intended for research use only.**
- The kit measures plasma unacylated ghrelin.
- Quality controls are rat-plasma based.

# 2. STORAGE, EXPIRATION

Store the complete kit at -20°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

# 3. INTRODUCTION

Ghrelin, an endogenous ligand for the growth hormone secretagogue receptor, is synthesized principally in the stomach. It stimulates food intake and transduces signals to hypothalamic regulatory nucleid that control energy homeostasis. The peptide consists of 28 amino acids, with a n-octanoylation of the serine-3 residue, which is necessary for the biological activity mentioned below. Ghrelin is present in the peripheral circulation under two forms: acylated and unacylated. The Rat Unacylated Ghrelin ELISA kit specifically measures the unacylated form of ghrelin.

**Areas of investigation:**
Animal studies, Energy metabolism and body weight regulation

## 4. TEST PRINCIPLE

The BioVendor Rat Unacylated Ghrelin ELISA is based on a double-antibody sandwich technique. The wells of the plate supplied with the kit are coated with a monoclonal antibody specific to the C-terminal part of ghrelin. This antibody will bind to any ghrelin introduced into the wells (standard or sample). The acetylcholinesterase (AChE) - Fab' conjugate which recognizes the N-terminal part of unacylated ghrelin is also added to the wells. This allows the two antibodies to form a sandwich by binding on different parts of the rat unacylated ghrelin.
The sandwich is immobilized on the plate so the excess reagents may be washed away. The concentration of the rat unacylated ghrelin is then determined by measuring the enzymatic activity of the immobilized AChE using the Ellman's Reagent. The AChE tracer acts on the Ellman's Reagent to form a yellow compound. The intensity of the color, which is determined by spectrophotometry, is proportional to the amount of the rat unacylated ghrelin present in the well during the immunological incubation.

## 5. PRECAUTIONS

- 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. However, these materials should be handled as potentially infectious.
- Avoid contact with the Substrate Solution (Ellman’s Reagent). In the case of contact, wash your skin thoroughly with water.
- 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.
- 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 | lyophilized | 1 vial |
| Rat Unacylated Ghrelin Standard | lyophilized | 2 vials |
| Quality Control | lyophilized | 2 vials |
| Dilution Buffer | lyophilized | 1 vial |
| Wash Solution Concentrate | concentrated | 1 vial |
| Tween 20 | ready to use | 1 vial |
| Substrate Solution (Ellman's reagent) | lyophilized | 2 vials |
| Product Data Sheet + Certificate of Analysis | | 1 pc |
| Template Sheet + Cover Sheet | | 1 pc |

## 8. MATERIAL REQUIRED BUT NOT SUPPLIED

- EDTA-plasma collection test tubes
- Test tubes for diluting samples (polypropylene)
- Precision pipettes to deliver 20-1000 µl and disposable tips
- Multichannel pipette 100-200 µl (recommended)
- Microplate reader with 405 or 414 nm filter
- Orbital microplate shaker
- Software package facilitating data generation and analysis
- Microtitration plate washer (optional) [Manual washing is possible but not preferable.]
- Glassware (graduated cylinder and bottle for Wash Solution)
- Deionized (distilled) water

## 9. PREPARATION OF REAGENTS

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

» **The kit can be used in two independent runs.**

» **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.

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

**Dilution Buffer**
Reconstitute one vial with 50 ml of distilled or deionized water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.
Stability and storage:
The reconstituted Dilution Buffer is stable 1 month when stored 4°C

**Rat Unacylated Ghrelin Standard**
Reconstitute one vial of the lyophilized Standard with 1 ml of distilled or deionized water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion. The concentration of the unacylated ghrelin standard S1 is 250 pg/ml.

Use seven polypropylene tubes to prepare the set of standards S2 to S8 from the reconstituted lyophilized standard S1 by serial dilution with Dilution Buffer as follows:

| *Standard to be prepared* | *Dilution Buffer* | *Standard to be added* |
|---|---|---|
| **S1** (250 pg/ml) | ----- | ----- |
| **S2** (125 pg/ml) | 500 µl | S1: 500 µl |
| **S3** (62.5 pg/ml) | 500 µl | S2: 500 µl |
| **S4** (31.3 pg/ml) | 500 µl | S3: 500 µl |
| **S5** (15.6 pg/ml) | 500 µl | S4: 500 µl |
| **S6** (7.81 pg/ml) | 500 µl | S5: 500 µl |
| **S7** (3.91 pg/ml) | 500 µl | S6: 500 µl |
| **S8** (1.96 pg/ml) | 500 µl | S7: 500 µl |

**Prepared Standards are ready to use, do not dilute them.**

Stability and storage:

Standard stock solution (250 pg/ml) should be aliquoted and frozen at –20°C for 1 week.
Do not store the diluted Standard solutions.

**Quality Control**

Reconstitute one vial with 1 ml of distilled or deionized water. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.

**Reconstituted Quality Control is ready to use, do not dilute it.**

Stability and storage:

The reconstituted Quality Control must be used immediately or aliquoted and frozen at -20°C for 1 week.

**Conjugate Solution (Anti-unacylated ghrelin-AChE tracer):**

Reconstitute one vial with 10 ml of Dilution Buffer. Allow it to stand 5 minutes until completely dissolved and then mix thoroughly by gentle inversion.

Stability and storage:

The reconstituted Conjugate Solution is stable 1 week when stored at 4°C

**Wash Solution**

Dilute 1 ml of Wash Solution Concentrate to 400 ml with distilled or deionized water. Add 200 µl of Tween 20 (use a magnetic stirrer to mix the contents).

Stability and storage:

The diluted Wash Solution is stable 1 week when stored at 4°C.

**Substrate Solution (Ellman's Reagent):**

Reconstitute one vial with 49 ml of distilled or deionized water and 1 ml of Wash Solution Concentrate five minutes before use. The tube contents should be thoroughly mixed.

Stability and storage:

The reconstituted Substrate Solution is stable 1 day when stored at 4°C and in the dark.

# 10. PREPARATION OF SAMPLES

The kit measures rat unacylated ghrelin in plasma.

Precautions!
All samples must be free of organic solvents prior to assay.
Samples should be assayed immediately after collection or should be stored at -20°C.

**Blood collection:**
Blood samples are collected in tubes containing EDTA. Samples are centrifuged at 3,500 rpm for 10 minutes at +4°C and then, supernatants are transferred in separate tubes. Samples should be quickly assayed or stored at -20°C for later use.

*For assaying the acylated ghrelin, please refer to the section "Blood collection" of the protocol of the RD394062400R Rat Acylated Ghrelin ELISA.*

**Sample preparation:**
Plasma samples may be directly assayed (without any extraction procedure) after being diluted at least to 10x in the Dilution Buffer in order to avoid matrix effect.

# 11. ASSAY PROCEDURE

1) Select strips sufficient for your assay and wash the wells 5-times with the prepared Wash Solution (0.3 ml per well) just before further using the strips.
   *Place the unused strips back in the packet and store at 4°C.*
2) Left four wells empty for blanking the Substrate Solution (Bl).
3) Pipette 100 µl of prepared Dilution buffer into four Non Specific Binding wells (NSB).
4) Pipette 100 µl of each of the eight prepared standards (S1 to S8) and Quality Control, preferably in duplicates into the respective wells.
5) Pipette 100 µl of diluted samples into the appropriate wells, preferably in duplicates (see example of work sheet).
6) Pipette 100 µl of Conjugate Solution to each well, except the Blank (Bl) wells.
7) Cover the plate with adhesive sheet.
8) Incubate the plate 3 hours at room temperature (ca. 25°C) or 20 hours at 4°C.
   *The long incubation period allows the increase of the assay sensitivity: 0.6 pg/ml versus 0.8 pg/ml for short incubation.*
9) Reconstitute the Substrate Solution.
10) Wash the wells 5-times with the Wash Solution (0.3 ml per well). Do not empty the wells after the fifth washing step, but shake the plate slightly for 5 minutes using an orbital shaker.
11) Repeat washing the wells 5-times with the wash buffer (0.3 ml per well).
12) Add 200 µl of Substrate Solution into each well including the blank (Bl) wells.
13) Incubate the plate in darkness at room temperature (using an orbital shaker is optimal for the color development).
14) Determine the absorbance by reading the plate at 405 to 414 nm: 30 minutes after adding the Substrate Solution for long first incubation period (20 hours at +4°C) or 30 to 60 minutes after adding the Substrate Solution for short first incubation period (3 hours at room temperature).

| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | Bl | S1 | S1 | QC | Sa | Sa | Sa | Sa | Sa | Sa | Sa | Sa |
| B | Bl | S2 | S2 | QC | Sa | Sa | Sa | Sa | Sa | Sa | Sa | Sa |
| C | Bl | S3 | S3 | Sa | Sa | Sa | Sa | Sa | Sa | Sa | Sa | Sa |
| D | Bl | S4 | S4 | Sa | Sa | Sa | Sa | Sa | Sa | Sa | Sa | Sa |
| E | NSB | S5 | S5 | Sa | Sa | Sa | Sa | Sa | Sa | Sa | Sa | Sa |
| F | NSB | S6 | S6 | Sa | Sa | Sa | Sa | Sa | Sa | Sa | Sa | Sa |
| G | NSB | S7 | S7 | Sa | Sa | Sa | Sa | Sa | Sa | Sa | Sa | Sa |
| H | NSB | S8 | S8 | Sa | Sa | Sa | Sa | Sa | Sa | Sa | Sa | Sa |

*Figure 1: Example of a work sheet.*

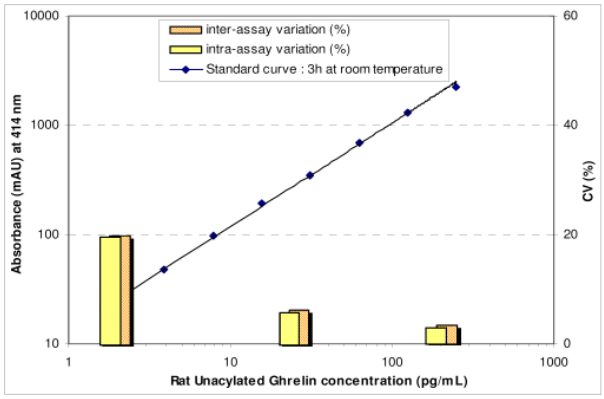
## 12. CALCULATIONS

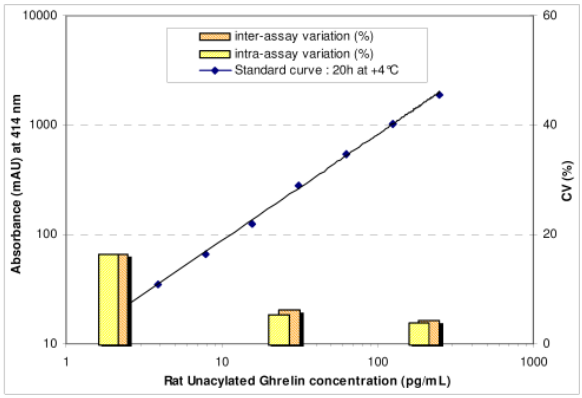
Most microtiter plate readers perform automatic calculations of analyte concentration. The calibration curve is constructed by plotting the absorbance (Y) of standards versus *log* of the known concentration (X) of standards, using the four-parameter function. The results are reported as the unacylated ghrelin concentration in samples (pg/ml). Make sure that your plate reader has subtracted the absorbance readings of the blank wells from absorbance readings of the rest of the plate. If not, do it manually.
Alternatively, the *logit log* function can be used to linearize the calibration curve (i.e. *logit* of absorbance (Y) is plotted versus *log* of the known concentration (X) of standards).
The actual concentration of unacylated ghrelin in the original plasma sample has been assessed by multiplying the assay result by the dilution factor 10 (e.g. 13.5 pg/ml x 10 gives 135 pg/ml).

## 13. PERFORMANCE CHARACTERISTICS

**Typical analytical data of BioVendor Rat Unacylated Ghrelin ELISA are presented in this chapter.**

The following data are for demonstration purpose only. Your data may be different and still correct. These data were obtained using all reagents as supplied in this kit under the following conditions: 30 minutes developing at room temperature for long first incubation period (20h at +4°C) and 60 minutes developing for short first incubation period (3h at RT), reading at 414 nm. A 4-parameter logistic fitting was used to determine the concentrations.



Example data:

| | *Absorbances* | |
|---|---|---|
| *Ghrelin standard (pg/ml)* | *Short first incubation period (3h RT)* | *Long first incubation period (20h +4°C)* |
| 250 | 2.220 | 1.888 |
| 125 | 1.307 | 1.033 |
| 62.5 | 0.697 | 0.548 |
| 31.3 | 0.351 | 0.277 |
| 15.6 | 0.195 | 0.126 |
| 7.81 | 0.097 | 0.066 |
| 3.91 | 0.048 | 0.035 |
| 1.95 | 0.022 | 0.019 |

- **Sensitivity**

The Rat Unacylated Ghrelin ELISA has been validated for its use in buffer and in plasma (without extraction but diluted at least 1:10). A sigmoidal 4-parameter logistic fitting was used to determine the concentrations.

The Limit of Determination, calculated as the concentration of unacylated ghrelin corresponding to the NSB average (n = 8) plus three standard deviations is 0.6 pg/ml and 0.8 pg/ml for long and short first incubation period, respectively.

The Limit of Quantification. Due to the minimal plasma dilution needed (1:10), the limits of quantification in the samples are 6 pg/ml (20h at +4°C) and 8 pg/ml (3h at RT), respectively.

- **Limit of assay**

Results exceeding concentration 250 pg/ml should be repeated with more diluted samples (e.g. 1:20). In this case, dilution factor need to be taken into consideration in calculating of the concentrations.
Non Specific Binding Absorbance <0.050 is acceptable.

- **Precision**

Intra-assay (Within-Run), Inter-assay (Run-to-Run) and Spiking Recovery

The intra-assay and inter-assay variations were studied on 30 rat plasma (free of ghrelin) spiked samples for each level of QC. QC´s were prepared as 10x concentrated from a pool of rat plasma and then diluted to 10x in Dilution Buffer before assay. Replicate samples (n=6) at each of the three validation levels were analyzed along with the calibration curve for a total of 5 independent runs.

| *Samples* | ***E**xpected concentrations in diluted QC (pg/ml)* | ***O**bserved concentrations (pg/ml)* | *Intra-assay CV (%)* | *Inter-assay CV (%)* | *Recovery **O/E** (%)* | *Confidence Interval ($\alpha$ = 0.05)* |
|---|---|---|---|---|---|---|
| Incubation 20 hours at +4°C | | | | | | |
| QC1 | 2 | 2.4 | 15.9 | 15.9 | 120 | 120 ± 7.3 |
| QC2 | 25 | 22.8 | 4.8 | 5.5 | 91.2 | 91.2 ± 2.6 |
| QC3 | 200 | 187 | 4.0 | 4.3 | 93.3 | 93.3 ± 1.9 |
| Incubation 3 hours at room temperature | | | | | | |
| QC1 | 2 | 2.34 | 19.5 | 19.8 | 117 | 117 ± 9.3 |
| QC2 | 25 | 22.7 | 5.2 | 5.5 | 90.6 | 90.6 ± 2.1 |
| QC3 | 200 | 184 | 2.9 | 3.3 | 91.8 | 91.8 ± 1.6 |

**Linearity**

Rat plasma samples were diluted 10x. Afterwards, four independent dilutions (n=3) were performed and the samples were assayed.

| *Samples* | *Dilution factor* | *Unacylated ghrelin measured (pg/ml)* | *Corrected Concentrations (pg/ml)* | *Recovery (%)* | *Mean Recovery (%)* |
|---|---|---|---|---|---|
| 1 | 10x | 79.2 | 792 | - | 98.9 |
| | 20x | 41.0 | 820 | 103 | |
| | 50x | 17.0 | 850 | 104 | |
| | 100x | 7.84 | 784 | 94.0 | |
| | 200x | 4.14 | 828 | 94.3 | |
| 2 | 10x | 86.8 | 868 | - | 101 |
| | 20x | 44.1 | 882 | 102 | |
| | 50x | 18.1 | 905 | 104 | |
| | 100x | 8.58 | 858 | 98.8 | |
| | 200x | 4.33 | 866 | 99.8 | |
| 3 | 10x | 83.6 | 836 | - | 96.7 |
| | 20x | 42.7 | 854 | 102 | |
| | 50x | 16.7 | 835 | 99.9 | |
| | 100x | 7.74 | 774 | 92.6 | |
| | 200x | 3.86 | 772 | 92.3 | |

- **Effect of Sample Matrix**

Five individual lots of rat plasma samples were tested. Validation samples (n=3) were prepared five times, concentrated in each matrix (free of ghrelin) and then diluted to 10x in order to obtain a final concentration of 25 pg/ml. Sample concentrations were read from a calibration curve derived from a pool of rat plasmas.

| Sample | Expected *(pg/ml)* | Observed *(pg/ml)* | Recovery *(%)* | Mean Recovery *(%)* |
|---|---|---|---|---|
| 1 | 25 | 25.9 | 104 | 103 |
| 2 | | 27.3 | 109 | |
| 3 | | 25.8 | 102 | |
| 4 | | 24.7 | 98.8 | |
| 5 | | 24.7 | 98.8 | |

- **Effect of Freezing/Thawing (sample stability)**

Five rat plasma samples (n=3) were analyzed just after collection and dilution to 10x before the assay (expected value) and after 1, 2 and 3 freeze/thaw cycles.

| Samples | **E**xpected value *(pg/ml)* | **O**bserved 1 cycle *(pg/ml)* | **O**bserved 2 cycles *(pg/ml)* | **O**bserved 3 cycles *(pg/ml)* | Mean recovery **O**/**E** *(%)* |
|---|---|---|---|---|---|
| 1 | 771 | 803 | 820 | 727 | 101.6 |
| 2 | 617 | 602 | 632 | 563 | 97.1 |
| 3 | 668 | 616 | 642 | 597 | 92.6 |
| 4 | 749 | 689 | 700 | 660 | 91.2 |
| 5 | 838 | 784 | 722 | 715 | 88.3 |

- **Specificity**

The Rat Unacylated Ghrelin ELISA is highly specific. The cross-reactivity values for related peptides have been as follows:

Cross-reactivity:
- Ghrelin (Des-Octanoyl-Ser$^3$) (Human): 100 %
- Ghrelin (Octanoyl-Ser$^3$) (Rat, Mouse): <0.001 %
- Ghrelin (Octanoyl-Ser$^3$) (Human): <0.001 %
- Ghrelin (1-11) (Rat): <0.001 %
- Ghrelin (1-14) (Human): <0.001 %
- Ghrelin (17-28) (Human, Rat): <0.001 %
- GHRF (Rat): <0.001 %
- Insulin (Rat): <0.001 %
- Motiline: <0.001 %
- Leptin (Rat): <0.001 %
- Somatostatine: <0.001 %
- CRF (Human, Rat): <0.001 %
- Glucagon (Human, Rat): <0.001 %

## 14. 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)

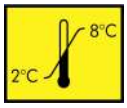
Possible explanation:

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

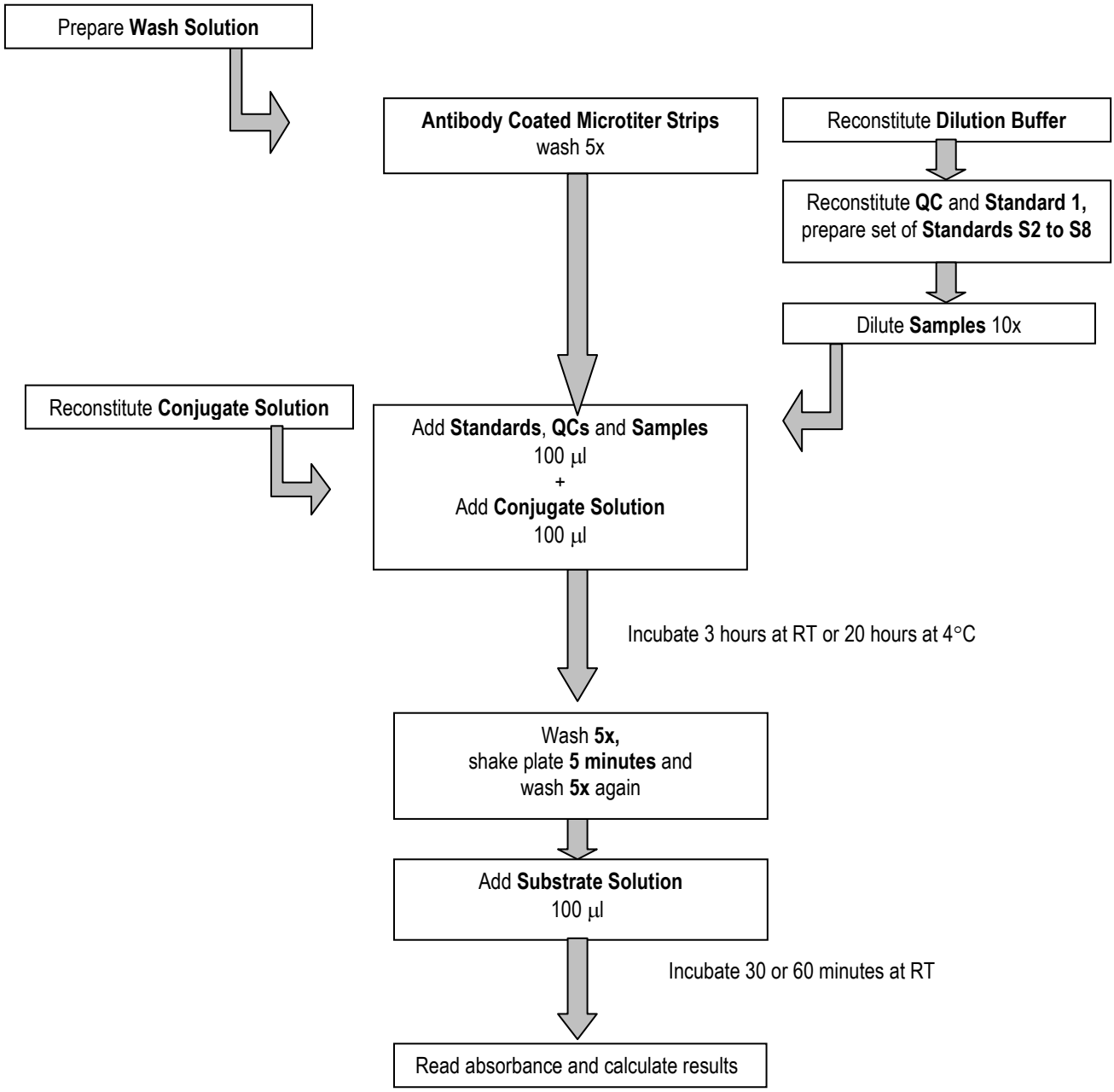
## 15. REFERENCES


- Grassi J. & Pradelles Ph. Compounds labelled by the acetylcholinesterase of Electrophorus Electricus. Its preparation process and it use as a tracer or marquer in enzymo-immunological determinations. *United States patent, N° 1,047,330. September 10, 1991*

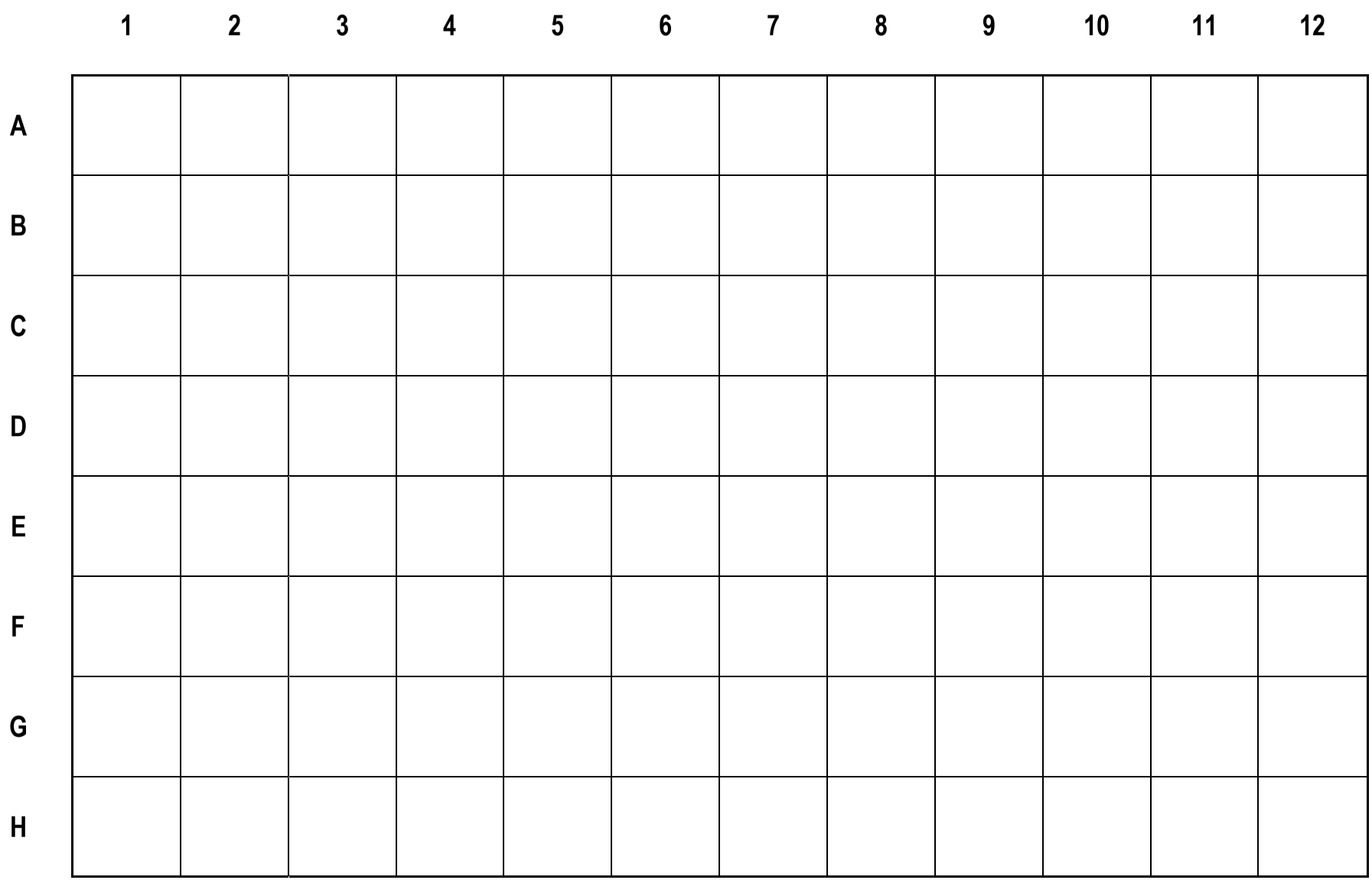
# 16. EXPLANATION OF SYMBOLS

| | |
|---|---|
| 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

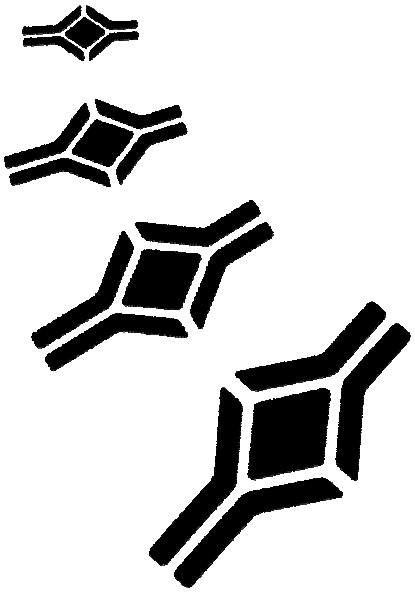



*The BioVendor RD394063400 Rat Unacylated Ghrelin ELISA is manufactured and distributed on agreement of the Société de Pharmacologie et d'Immunologie - BIO, Parc d'activités du Pas du Lac 10 bis, Avenue Ampère, F-78180 Montigny le Bretonneux, FRANCE*


1
2
3
4
5
6
7
8
9
10
11
12
A
B
C
D
E
F
G
H

## NOTES

BioVendor
Research
and Diagnostic Products




| | | | | |
|---|---|---|---|---|
| **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: +86-20-8706-3029<br>Fax: +86-20-8706-3016 | E-mail: infoCN@biovendor.com |