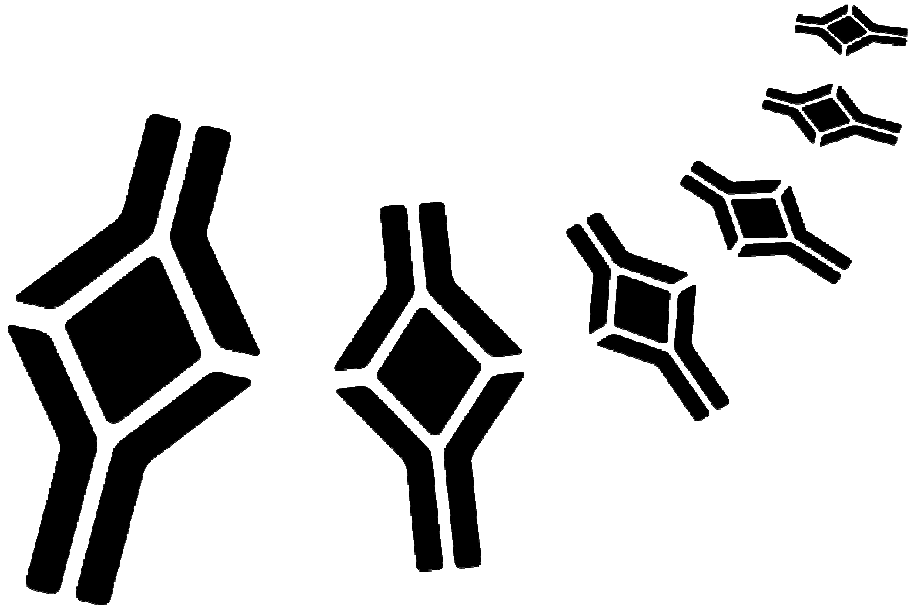
BioVendor
Research
and Diagnostic Products



# HUMAN LEPTIN ELISA, Clinical Range

## Product Data Sheet

## Cat No.: RD191001100

European Union: IVD CE

Rest of the world:
For research use only!

# 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 RD191001100 Human Leptin ELISA, Clinical Range is a sandwich enzyme immunoassay for the quantitative measurement of human leptin.

## Features

- **European Union: for in vitro diagnostic use**
  **Rest of the world: for research use only!**
- The total assay time is less than 2.5 hours
- The kit measures total leptin in serum and plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- Quality Controls are human serum based
- Standards are 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

Leptin, the product of the *ob* (obese) gene, is a single-chain 16 kDa protein consisting of 146 amino acid residues. Leptin is produced mainly in the adipose tissue, and is considered to play an important role in appetite control, fat metabolism and body weight regulation. It targets the central nervous system, particularly hypothalamus, affecting food intake. The primary effect of leptin appears to be mediated by leptin receptors expressed mainly in the hypothalamus. In humans, leptin levels correlate with body mass index (BMI) and percentage body fat, and are elevated even in obese individuals. Leptin has a dual action; it decreases the appetite and increases energy consumption, causing more fat to be burned. Leptin is secreted in circadian fashion with nocturnal rise in both lean and obese patients.
Mutations of the *ob* gene resulting in leptin deficiency are the cause of obesity in the *ob/ob* mice. Endogeneous leptin can normalize their body weight. In contrast, high levels of leptin in obese human subjects point to an insensitivity to endogeneous leptin.
Other factors in addition to the amount of body fat appear to regulate leptin action: insulin, glucocorticoids, catecholamines and sex hormones. Studies have shown that leptin may be linked to reproductive function.

Areas of investigation:
Energy metabolism and body weight regulation

# 4. TEST PRINCIPLE

In the BioVendor Human Leptin ELISA Clinical Range, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human leptin antibody. After 60 minutes incubation and washing, polyclonal anti-human leptin antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with captured leptin. 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. The absorbance is proportional to the concentration of leptin. 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 |
| Conjugate Solution | ready to use | 13 ml |
| Master Standard | lyophilized | 1 vial |
| Quality Control HIGH | lyophilized | 1 vial |
| Quality Control LOW | lyophilized | 1 vial |
| 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, 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 desicant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8°C and protected from the moisture.

**Conjugate solution**
**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 Leptin Master Standard**
**Refer to the Certificate 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 leptin in the stock solution is **50 ng/ml.**

Prepare set of standards using Dilution Buffer as follows:

| *Volume of Standard* | *Dilution Buffer* | *Concentration* |
|---|---|---|
| Stock | - | 50 ng/ml |
| 200 µl of stock | 300 µl | 20 ng/ml |
| 250 µl of 20 ng/ml | 250 µl | 10 ng/ml |
| 250 µl of 10 ng/ml | 250 µl | 5 ng/ml |
| 200 µl of 5 ng/ml | 300 µl | 2 ng/ml |
| 250 µl of 2 ng/ml | 250 µl | 1 ng/ml |

The volume of dilution buffer for reconstitution of Master standard given in CoA dilutes standards 3x, the same as samples and controls.

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

Stability and storage:

The reconstituted Master Standard must be used immediately or stored frozen at –20 °C for 3 months. Avoid repeating freezing/thawing cycles.

**Do not store the diluted Standard solutions.**

**Quality Controls HIGH, LOW**

**Refer to the Certificate of Analysis for current Quality Control concentration!!!**

Reconstitute each Quality Control (HIGH and LOW) with 350 µl of distilled water just prior to the assay. Let it dissolve at least 30 minutes with occasional gentle shaking (not to foam).

Dilute reconstituted Quality Controls 3x with Dilution Buffer, e.g. 50 µl of Quality Control + 100 µl of Dilution Buffer when assaying samples in singlets, or preferably 100 µl of Quality Control + 200 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 kit works in accordance with PDS and CoA and that ELISA test was carried out properly.*

**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 leptin in serum or plasma (EDTA, citrate, heparin).

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 serum or plasma samples 3x with Dilution Buffer just prior to the assay, e.g. 50 µl of sample + 100 µl of Dilution Buffer for singlets, or preferably 100 µl of sample + 200 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:
Samples should be stored at -20°C, 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 serum and plasma samples when stored at 2-8°C, effect of freezing/thawing and effect of sample matrix (serum/plasma) on the concentration of leptin.

*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 information at info@biovendor.com if assaying tissue culture supernatants.

# 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 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 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 9.**

*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 leptin 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 50** | **Blank** | Sample 8 | Sample 16 | Sample 24 | Sample 32 |
| **B** | **Standard 20** | Sample 1 | Sample 9 | Sample 17 | Sample 25 | Sample 33 |
| **C** | **Standard 10** | Sample 2 | Sample 10 | Sample 18 | Sample 26 | Sample 34 |
| **D** | **Standard 5** | Sample 3 | Sample 11 | Sample 19 | Sample 27 | Sample 35 |
| **E** | **Standard 2** | Sample 4 | Sample 12 | Sample 20 | Sample 28 | Sample 36 |
| **F** | **Standard 1** | 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 microtiter plate 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 leptin 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.

**Set of Standards are diluted 3x during reconstitution with the specified volume of buffer and Samples and Quality Controls are all diluted 3x prior to analysis, so there is no need to take this dilution factor into account.**

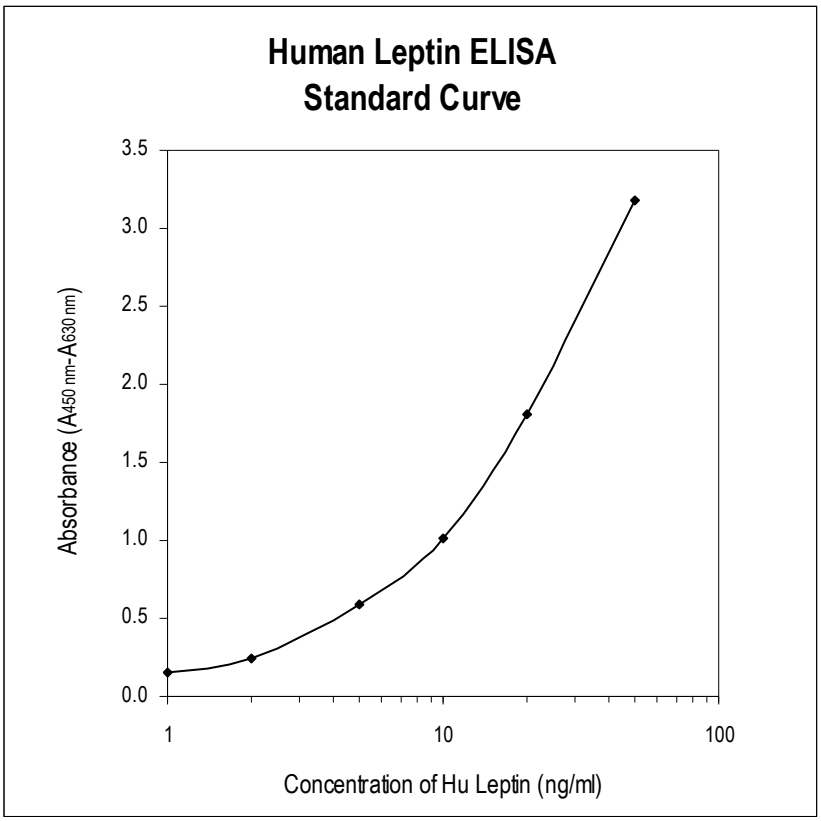



*Figure 2: Typical Standard Curve for Human Leptin ELISA, Clinical Range.*

# 13. PERFORMANCE CHARACTERISTICS

**Typical analytical data of BioVendor Human Leptin ELISA, Clinical Range 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 leptin values in wells and is 0.2 ng/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding leptin level of 50 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the leptin concentration.

- **Specificity**

The antibodies used in this ELISA are specific for human leptin.
Determination of leptin does not interfere with hemoglobin (1.0 mg/ml), bilirubin (170 umol/l) and triglycerides (5.0 mmol/l).

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 |
| Monkey | no |
| 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 | 15.01 | 0.06 | 4.2 |
| 2 | 3.56 | 0.02 | 7.6 |

Inter-assay (Run-to-Run) (n=6)

| *Sample* | *Mean (ng/ml)* | *SD (ng/ml)* | *CV (%)* |
|---|---|---|---|
| 1 | 15.39 | 1.04 | 6.7 |
| 2 | 29.34 | 1.28 | 4.4 |

- **Spiking Recovery**

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

| *Sample* | ***O**bserved (ng/ml)* | ***E**xpected (ng/ml)* | *Recovery **O/E** (%)* |
|---|---|---|---|
| 1 | 4.22 | - | - |
| | 7.52 | 8.40 | 89.5 |
| | 11.85 | 13.37 | 88.6 |
| | 17.41 | 17.76 | 98.0 |
| 2 | 14.09 | - | - |
| | 17.78 | 18.27 | 97.3 |
| | 19.92 | 23.24 | 85.7 |
| | 25.91 | 27.63 | 93.8 |

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

| *Sample* | *Dilution* | ***O**bserved (ng/ml)* | ***E**xpected (ng/ml)* | *Recovery **O/E** (%)* |
|---|---|---|---|---|
| 1 | - | 20.99 | - | - |
| | 2x | 10.34 | 10.49 | 98.5 |
| | 4x | 5.32 | 5.25 | 101.4 |
| | 8x | 2.45 | 2.62 | 93.4 |
| 2 | - | 29.94 | - | - |
| | 2x | 15.72 | 14.97 | 105.0 |
| | 4x | 7.80 | 7.49 | 104.2 |
| | 8x | 3.83 | 3.74 | 102.3 |

- **Effect of sample matrix**

Citrate, heparin and EDTA plasmas were compared to respective serum samples from the same 9 individuals. Results are shown below:

| *Volunteer No.* | *Serum (ng/ml)* | *Plasma (ng/ml)* | | |
|---|---|---|---|---|
| | | *EDTA* | *Citrate* | *Heparin* |
| 1 | 7.72 | 7.41 | 6.47 | 7.13 |
| 2 | 9.05 | 7.84 | 6.57 | 8.95 |
| 3 | 2.54 | 2.18 | 1.81 | 2.32 |
| 4 | 7.08 | 6.13 | 5.97 | 7.47 |
| 5 | 18.71 | 16.94 | 13.81 | 17.55 |
| 6 | 19.64 | 16.01 | 15.05 | 23.39 |
| 7 | 6.42 | 6.31 | 5.65 | 6.76 |
| 8 | 3.97 | 3.93 | 3.32 | 3.36 |
| 9 | 5.67 | 7.17 | 6.38 | 5.84 |
| **Mean (ng/ml)** | **8.97** | **8.21** | **7.22** | **9.19** |
| **Mean Plasma/Serum (%)** | **-** | **91.6** | **80.6** | **102.7** |
| **Coefficient of determination $R^2$** | **-** | **0.97** | **0.97** | **0.96** |

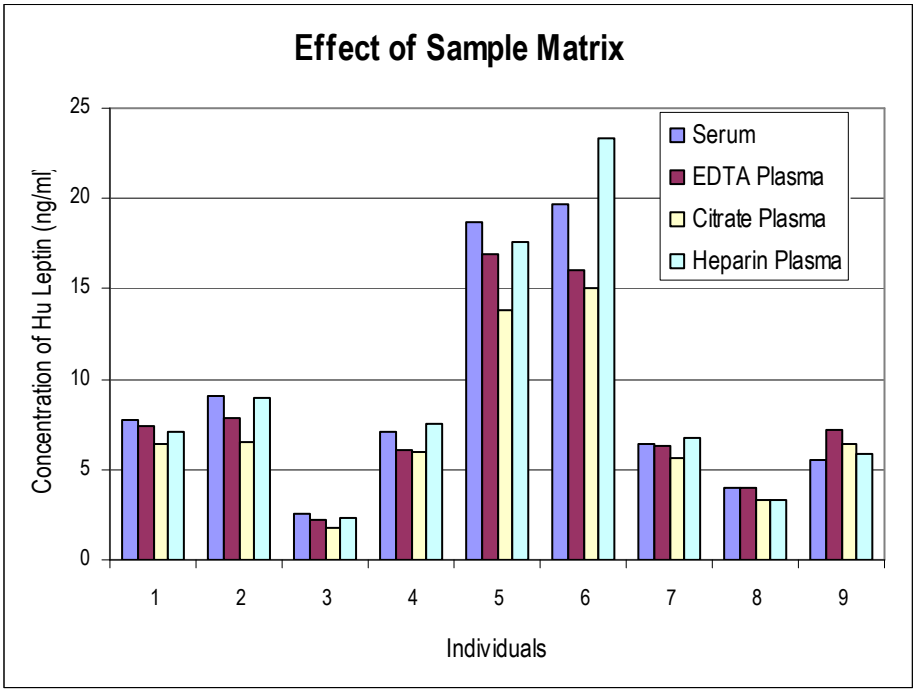



*Figure 3: Leptin levels measured using Human Leptin ELISA, Clinical Range from 9 individuals using serum, EDTA, citrate and heparin plasma, respectively.*

- **Stability of samples stored at 2-8°C**

Samples should be stored at –20°C. However, no decline in concentration of leptin was observed in serum and plasma samples after 7 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* | *Serum (ng/ml)* | *Plasma (ng/ml)* | | |
|---|---|---|---|---|---|
| | | | *EDTA* | *Citrate* | *Heparin* |
| 1 | -20°C | 2.03 | 2.21 | 1.79 | 1.90 |
| | 2-8°C, 1 day | 2.18 | 2.21 | 1.83 | 1.00 |
| | 2-8°C, 7 days | 2.26 | 2.21 | 1.64 | 2.28 |
| 2 | -20°C | 3.51 | 3.54 | 3.26 | 3..56 |
| | 2-8°C, 1 day | 3.65 | 3.79 | 3.42 | 2.95 |
| | 2-8°C, 7 days | 3.74 | 3.49 | 3.19 | 4.09 |
| 3 | -20°C | 8.76 | 9.20 | 7.13 | 8.94 |
| | 2-8°C, 1 day | 7.80 | 8.99 | 8.19 | 8.56 |
| | 2-8°C, 7 days | 7.70 | 8.03 | 7.87 | 8.43 |

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human leptin in serum and plasma samples after repeated (5x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

| *Sample* | *Number of f/t cycles* | *Serum (ng/ml)* | *Plasma (ng/ml)* | | |
|---|---|---|---|---|---|
| | | | *EDTA* | *Citrate* | *Heparin* |
| 1 | 1x | 5.90 | 6.05 | 5.23 | 5.63 |
| | 3x | 5.78 | 5.49 | 5.40 | 5.39 |
| | 5x | 5.64 | 5.99 | 5.47 | 6.14 |
| 2 | 1x | 3.09 | 3.29 | 2.85 | 2.86 |
| | 3x | 3.21 | 3.28 | 2.81 | 2.71 |
| | 5x | 3.44 | 3.41 | 2.72 | 3.54 |
| 3 | 1x | 4.63 | 5.33 | 4.71 | 4.80 |
| | 3x | 3.73 | 4.96 | 4.67 | 4.61 |
| | 5x | 4.58 | 5.39 | 4.80 | 4.91 |

- **Reference range**

It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and pathological reference ranges for leptin levels with the assay.

## 14. DEFINITION OF THE STANDARD

A recombinant protein is used as the standard. The recombinant human leptin is a 16 kDa protein containing 147 amino acid residues.

## 15. METHOD COMPARISON

The BioVendor Human Leptin ELISA, Clinical Range was compared to a commercial RIA. Linear regression analysis of the results yielded the following results.

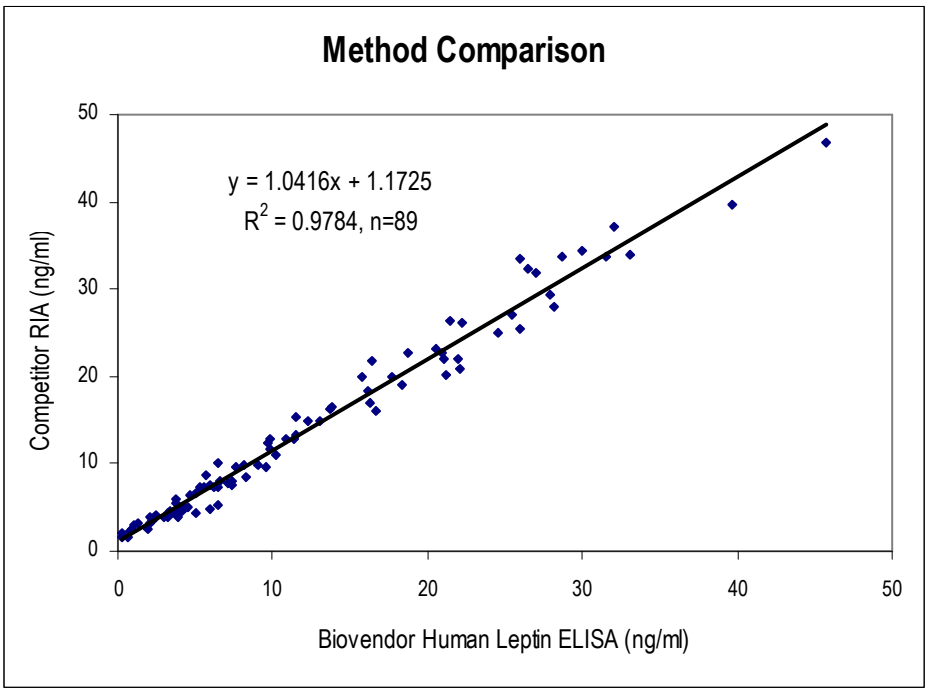



*Figure 4: Method comparison.*

# 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 leptin:

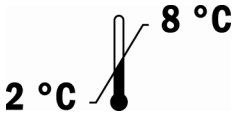

- Auwerx J. and Staels B.: Leptin (Review article). The Lancet 13, 737 (1998)
- Blum W.F., Englaro P., Hanitsch S. et al.: Plasma leptin levels in healthy children and adolescents: Dependence on body mass index, body fat mass, gender, pubertal stage, and testosterone. J. Clin. Endocrinol. Metab. 82, 2904 (1997)
- Cohen B., Novick D. and Rubinstein M.: Modulation of insulin activities by leptin. Science 274, 1185 (1996)
- Considine R.V., Sinha M.K., Heiman M.L., Kriaciunas A., Stephens T.W., Nyce M.R., Ohannesian J.P., Marco C.C., McKee L.J., Bauer T.L. and Caro J.F.: Serum immunoreactive leptin concentrations in normal-weight and obese humans. N. Engl. J. Med. 334, 292-295 (1996)
- Halaas J.L., Gajiwala K.S., Maffei M., Cohen S.L., Chait B.T., Rabinowitz D., Lallone R.L., Burley S.K. and Friedman J.M.: Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269, 543-546 (1995)
- Harigaya A., Nagashima K., Nako Y. and Marikawa A.: Relationship between concentration of serum leptin and fetal growth. J. Clin. Endocrinol. Metab. 82, 3281 (1997)
- Lönnqvist F., Arner P., Nordfors L. and Shalling M.: Overexpression of the obese (ob) gene in adipose tissue of human subjects. Nature Med. 1, 950-953 (1995)
- Maffei M., Halaas J., Ravussin E., Pratley R.E., Lee G.H., Zhang Y., Fei H., Kim S., Lallone R., Ranganathan S., Kern P.A. and Friedman J.M.: Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nature Med. 1, 1155-1161 (1995)
- Pelleymounter M.A., Cullen M.J., Baker M.B., Hecht R., Winters D., Boone T. and Collins F.: Effects of the obese gene product on body weight regulation in ob/ob mice. Science 269, 540-543 (1995)
- Spiegelman B.M., Flier J.S.: Adipogenesis and obesity: Rounding out the big picture, Cell 87, 377-389 (1996)
- Tartaglia L.A.: The leptin receptor, J. Biol. Chem. 272, 6093-6096 (1997)
- Tritos N.A., Mantzoros C.S.: Leptin: its role in obesity and beyond, Diabetologia 40, 1371-1379 (1997)
- Zhang F., Basinski M. B., Beals J.M., Briggs S.L., Churgay L.M., Clawson D.K., DiMarchi R.D., Furman T.C., Hale J.E., Hsiung H.M., Schoner B.E., Smith D.P., Zhang X.Y., Wery J.P., and Schevitz R.W.: Crystal structure of the obese protein leptin-E100, Nature 387, 206-209 (1997)
- Zhang Y., Proenca R., Maffei M., Barone M., Leopold L., Friedman J.M.: Positional cloning of the mouse obese gene and its human homologue. Nature 372, 425-432 (1994)

## References to this product:

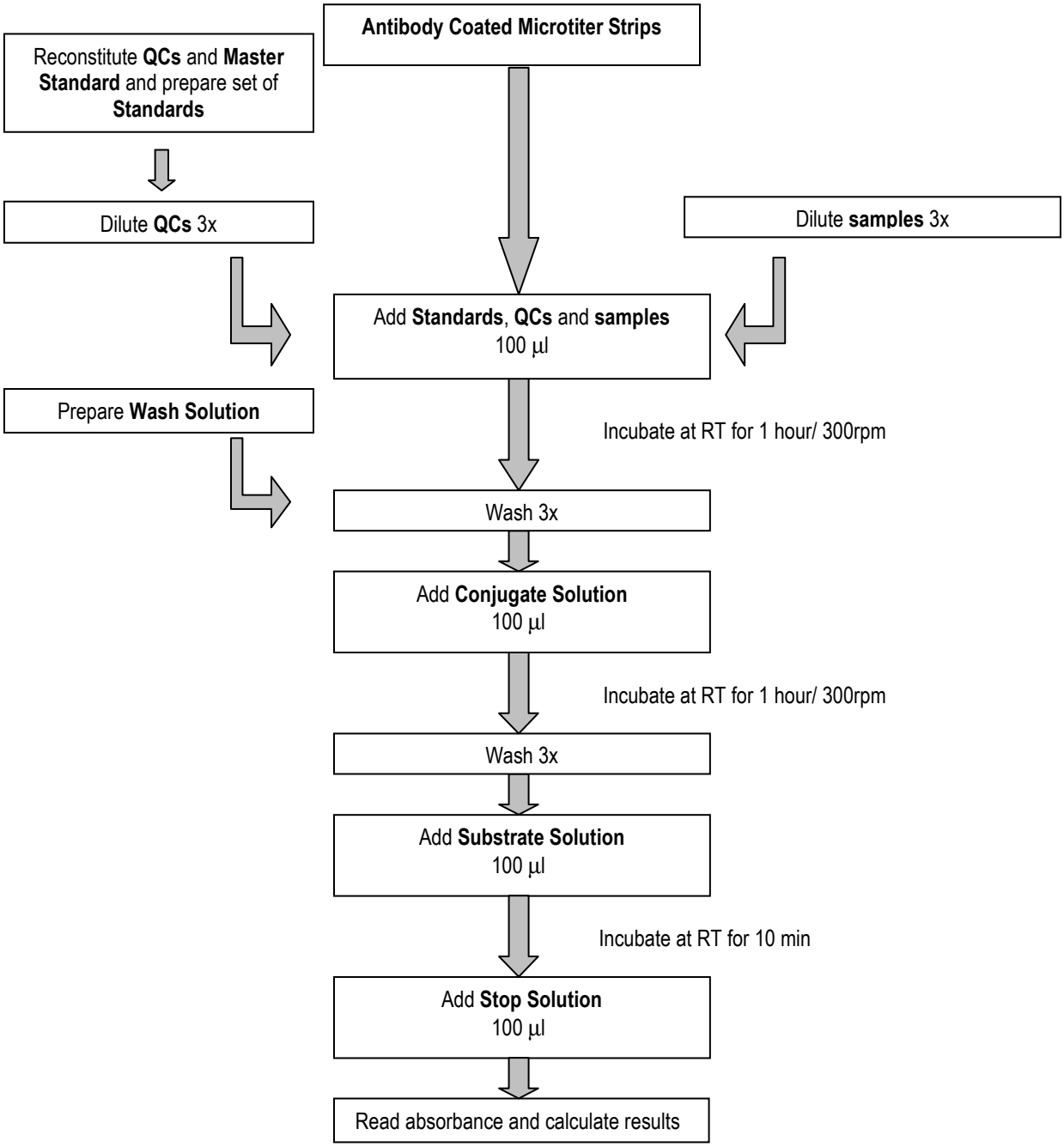

- Arnold T, Brandlhofer S, Vrtikapa K, Stangl H, Hermann M, Zwiauer K, Mangge H, Karwautz A, Huemer J, Koller D, Schneider WJ, Strobl W. Effect of obesity on plasma clusterin: a proposed modulator of leptin action. Pediatr Res. 2011 Mar;69 (3):237-42
- Azzoni L, Crowther NJ, Firnhaber C, Foulkes AS, Yin X, Glencross D, Gross R, Kaplan MD, Papasavvas E, Schulze D, Stevens W, van der Merwe T, Waisberg R, Sanne I, Montaner LJ. Association between HIV replication and serum leptin levels: an observational study of a cohort of HIV-1-infected South African women. J Int AIDS Soc. 2010;13 (1):33
- Aeberli I, Beljean N, Lehmann R, l'Allemand D, Spinas GA, Zimmermann MB. The increase of fatty acid-binding protein aP2 in overweight and obese children: interactions with dietary fat and impact on measures of subclinical inflammation. Int J Obes (Lond). 2008 Oct;32 (10):1513-20
- Risch L, Saely C, Hoefle G, Rein P, Langer P, Gouya G, Marte T, Aczel S, Drexel, H: Relationship between glomerular filtration rate and the adipokines adiponectin, resistin and leptin in coronary patients with predominantly normal or mildly impaired renal function. Clin Chim Acta 376 (1-2):108-13 (2007). Epub Jul 29 (2006)
- Bronsky J, Karpisek M, Bronska E, Pechova M, Jancikova B, Kotolova H, Stejskal D, Prusa R, Nevoral J: Adiponectin, adipocyte fatty acid binding protein, and epidermal fatty acid binding protein: proteins newly identified in human breast milk. Clin Chem 52 (9):1763-70 (2006)
- Lichnovska R, Gwozdziewiczova S, Chlup R, Hrebicek J.: Serum Leptin in the Development of Insulin Resistance and Other Disorders in the Metabolic Syndrome. Biomed. Papers 149 (1): 119-126 (2005)
- Meier U, and Gressner A.M.: Endocrine Regulation of Energy Metabolism: Review of Pathobiochemical and Clinical Chemical Aspects of Leptin, Ghrelin, Adiponectin, and Resistin. Clin Chem 50 (9): 1511-1525 (2004)
- Lichnovska R, Gwozdziewiczova S, Hrebicek J.: Gender differences in factors influencing insulin resistance in elderly hyperlipemic non-diabetic subjects. Cardiovasc Diabetol Oct 14;1(1):4 (2002)
- Adam JA, Menheere PP, van Dielen FM, Soeters PB, Buurman WA, Greve JW: Decreased plasma orexin-A levels in obese individuals. Int J Obes Relat Metab Disord 26 (2):274-6 (2002)
- Haluzik M, Sindelka G, Widimsky J Jr, Prazny M, Zelinka T, Skrha J.: Serum leptin levels in patients with primary hyperaldosteronism before and after treatment: relationships to insulin sensitivity. J Hum Hypertens 16 (1):41-5 (2002)

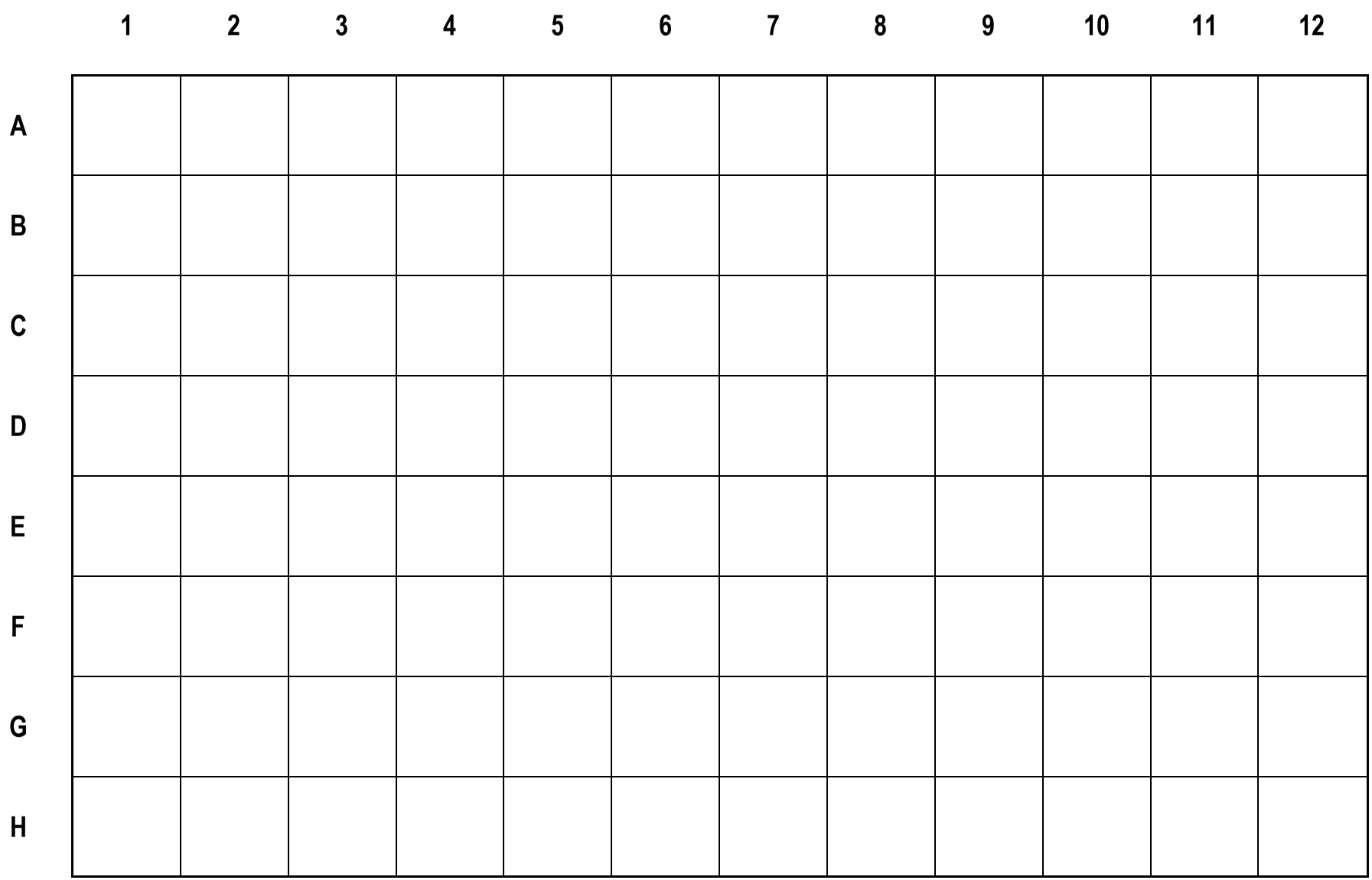

## For more references on this product see our WebPages at www.biovendor.com

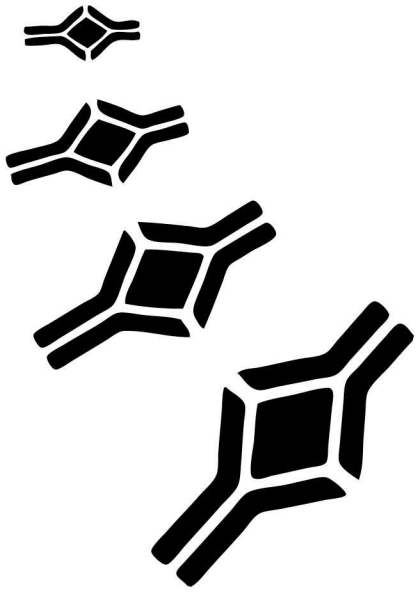
## 18. EXPLANATION OF SYMBOLS

| | |
|---|---|
| REF | Catalogue number |
| Cont. | Content |
| LOT | Lot number |
|  | See instructions for use |
|  | Biological hazard |
|  | Expiry date |
|   | Storage conditions |
|   | Identification of packaging materials |
| IVD CE | In vitro diagnostic medical device |

## Assay Procedure Summary




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



**BioVendor – Laboratorní medicína a.s.**
Karasek 1767/1, 621 00 Brno, Czech Republic
Phone: +420-549-124-185, Fax: +420-549-211-460
E-mail: info@biovendor.com, sales@biovendor.com
Web: www.biovendor.com

There are BioVendor branches and distributors near you.
To find the office closest to you, visit **www.biovendor.com/contact**

BioVendor
Research
and Diagnostic Products