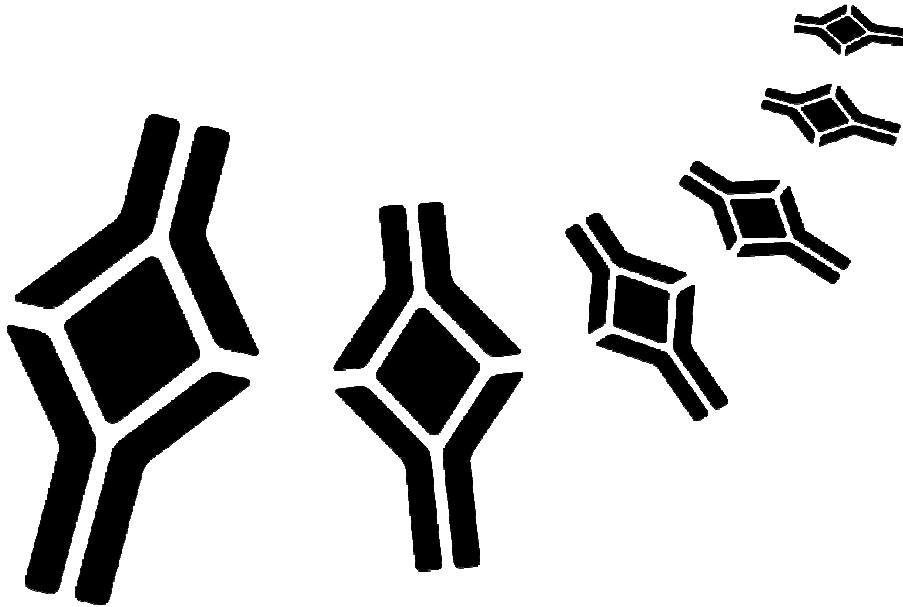


BioVendor

Research
and Diagnostic Products



HUMAN LEPTIN RECEPTOR ELISA

Product Data Sheet

Cat. No.: RD194002100

For Research Use Only

CONTENTS

1.	INTENDED USE	3
2.	STORAGE, EXPIRATION	3
3.	INTRODUCTION	4
4.	TEST PRINCIPLE	4
5.	PRECAUTIONS	5
6.	TECHNICAL HINTS	5
7.	REAGENT SUPPLIED	6
8.	MATERIAL REQUIRED BUT NOT SUPPLIED	6
9.	PREPARATION OF REAGENTS	7
10.	PREPARATION OF SAMPLES	8
11.	ASSAY PROCEDURE	10
12.	CALCULATIONS	12
13.	PERFORMANCE CHARACTERISTICS	13
14.	DEFINITION OF THE STANDARD	17
15.	METHOD COMPARISON	17
16.	TROUBLESHOOTING AND FAQs	18
17.	REFERENCES	19
18.	EXPLANATION OF SYMBOLS	24

**»» 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 RD194002100 Human Leptin Receptor ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human leptin receptor.

»» Features

- **It is intended for research use only**
- The total assay time is less than 2.5 hours
- The kit measures leptin receptor in serum and plasma (EDTA and heparin)
- Assay format is 96 wells
- Quality Controls are human serum based
- 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

Leptin receptor (OB-R) was identified as a leptin binding protein (leptin, the product of the *ob* gene, is a single-chain 16 kDa protein consisting of 146 amino acid residues.) OB-R was found to be a member of the class I cytokine receptor family with a large extracellular domain. Leptin receptor exists in multiple forms with a common extracellular domain and a variable length cytoplasmic portion. Alternate splicing from a single gene derives the six isoforms of the leptin receptor.

The soluble form of the leptin receptor, OB-R contains no intracellular motifs or transmembrane residues, thus it consists entirely of the extracellular ligand-binding domain of the receptor.

Long forms of OB-R transcripts were reported to be expressed predominantly in regions of the hypothalamus which provides evidence that leptin receptor is important in body weight regulation. Expression of short forms of OB-R transcripts have been found in multiple tissues, including the choroid plexus, lung, kidney, and primitive hematopoietic cell populations. Leptin receptor may act as a negative regulator of leptin activity and it may maintain a pool of available bioactive leptin by binding and delaying its clearance from circulation.

Soluble leptin receptor levels are indirectly proportional to adiposity and are increased in females versus males. Leptin receptor levels are highest in infants, decrease into adolescence, and remain relatively stable throughout adulthood. Soluble leptin receptor is also found upregulated in patients with chronic heart failure, end-stage renal disease and anorexia.

Areas of investigation:

Energy metabolism and body weight regulation

4. TEST PRINCIPLE

In the BioVendor Human Leptin Receptor ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with monoclonal anti-human leptin receptor antibody. After 60 minutes incubation and washing, monoclonal anti-human leptin receptor antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with captured leptin receptor. 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 receptor. 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. 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

<i>Kit Components</i>	<i>State</i>	<i>Quantity</i>
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	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 μ l with disposable tips
- Multichannel pipette to deliver 100 μ l with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtiter 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 desiccant and seal carefully. Remaining Microtiter Strips are stable 3 month when 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 month when stored at 2-8°C.

- Assay reagents supplied concentrated or lyophilized:

Human Leptin Receptor Master Standard

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution!!!

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 human leptin receptor in the stock solution is **32 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

<i>Volume of Standard</i>	<i>Dilution Buffer</i>	<i>Concentration</i>
Stock	-	32 ng/ml
250 µl of stock	250 µl	16 ng/ml
250 µl of 16 ng/ml	250 µl	8 ng/ml
250 µl of 8 ng/ml	250 µl	4 ng/ml
250 µl of 4 ng/ml	250 µl	2 ng/ml
250 µl of 2 ng/ml	250 µl	1 ng/ml

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

Stability and storage:

Do not store the stock solution, neither prepared 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 μ l of distilled (deionized) water just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).

Dilute reconstituted Quality Controls 3x with Dilution Buffer, e.g. 50 μ l of Quality Control + 100 μ l of Dilution Buffer when assaying samples in singlets, or preferably 100 μ l of Quality Control + 200 μ l of Dilution Buffer for duplicates. **Mix well** (not to foam).

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.

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

Stability and storage:

Samples should be stored at -20°, or preferably at -70°C for long-term storage. Avoid repeated freeze/ thaw cycles.

Do not store the diluted samples.

See Chapter 13 for stability of 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 receptor.

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 detail information at info@biovendor.com if assaying other type of samples.

11. ASSAY PROCEDURE

1. Pipet **100 µl** of Standards, Quality Controls, Dilution Buffer (=Blank) and diluted 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 5-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 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add **100 µl** of 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 receptor 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 four times. 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 32	Blank	Sample 8	Sample 16	Sample 24	Sample 32
B	Standard 16	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	Standard 8	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	Standard 4	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 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 leptin receptor (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 Quality Controls and samples calculated from the standard curve must be multiplied by their respective dilution factor because they have been diluted prior to the assay, e.g. 4 ng/ml (from standard curve) x 3 (dilution factor) = 12 ng/ml.

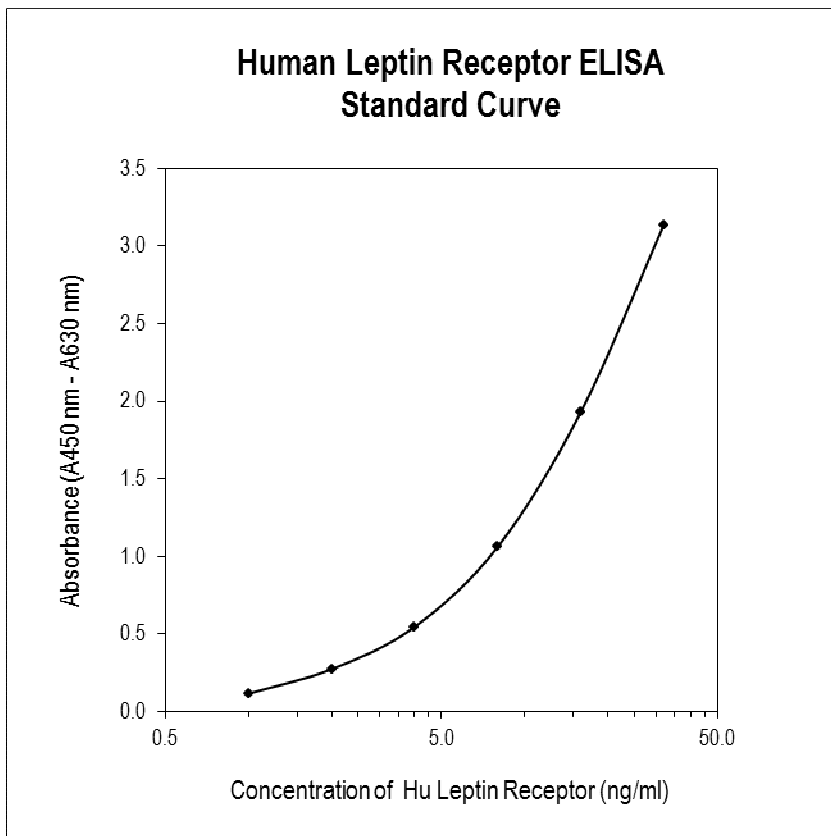


Figure 2: Typical Standard Curve for Human Leptin Receptor ELISA.

13. PERFORMANCE CHARACTERISTICS

➤➤ Typical analytical data of BioVendor Human Leptin Receptor 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: $A_{\text{blank}} + 3 \times \text{SD}_{\text{blank}}$) is calculated from the real leptin receptor values in wells and is 0.05 ng/ml.

*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Samples with absorbances exceeding the absorbance of the highest standard should be measured again with higher dilution. The final concentration of sample calculated from the standard curve must be multiplied by the respective dilution factor.

- **Specificity**

The antibodies used in this ELISA are specific for human leptin receptor with no detectable crossreactivities to human cytokines. Determination of leptin receptor does not interfere with haemoglobin (1.0 mg/ml), bilirubin (170 $\mu\text{mol/l}$) 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.

<i>Mammalian serum sample</i>	<i>Observed crossreactivity</i>
Bovine	no
Cat	no
Dog	no
Goat	no
Horse	no
Monkey	no
Mouse	no
Pig	no
Rabbit	no
Rat	no
Sheep	no

- **Precision**

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

<i>Sample</i>	<i>Mean (ng/ml)</i>	<i>SD (ng/ml)</i>	<i>CV (%)</i>
1	17.35	1.25	7.23
2	30.82	2.19	7.10

Inter-assay (Run-to-Run) (n=5)

<i>Sample</i>	<i>Mean (ng/ml)</i>	<i>SD (ng/ml)</i>	<i>CV (%)</i>
1	12.24	1.20	9.81
2	30.92	1.92	6.21

- **Spiking Recovery**

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

<i>Sample</i>	<i>Observed (ng/ml)</i>	<i>Expected (ng/ml)</i>	<i>Recovery O/E (%)</i>
1	10.75	-	-
	15.30	15.92	96.1
	19.54	22.68	86.2
	21.14	25.97	81.4
2	16.52	-	-
	20.62	21.69	95.1
	25.23	28.45	88.7
	26.64	31.74	83.9

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

<i>Sample</i>	<i>Dilution</i>	<i>Observed (ng/ml)</i>	<i>Expected (ng/ml)</i>	<i>Recovery O/E (%)</i>
1	-	34.57	-	-
	2x	19.49	17.29	112.8
	4x	10.03	8.64	116.1
	8x	4.30	4.32	99.5
2	-	28.15	-	-
	2x	14.78	14.08	105.0
	4x	8.01	7.04	113.8
	8x	3.52	3.52	100.0

- **Effect of sample matrix**

EDTA, citrate and heparin plasmas were compared to respective serum samples from the same 10 individuals. However, we observed low correlation among serum and citrate plasma leptin receptor values. Results are shown below:

Volunteer No.	Serum (ng/ml)	Plasma (ng/ml)		
		EDTA	Citrate	Heparin
1	32.73	31.68	29.91	31.54
2	34.32	36.19	35.55	34.39
3	50.14	39.93	36.81	42.49
4	22.56	24.76	26.17	27.44
5	26.58	24.50	17.08	28.06
6	22.31	21.55	23.02	23.37
7	28.11	24.99	23.06	26.04
8	22.20	22.81	22.77	24.57
9	31.81	28.62	23.15	30.46
10	22.78	25.69	20.82	23.69
Mean (ng/ml)	29.35	28.07	25.83	29.21
Mean Plasma/Serum (%)	-	95.6	92.0	113.1
Coefficient of determination R²	-	0.86	0.59	0.93

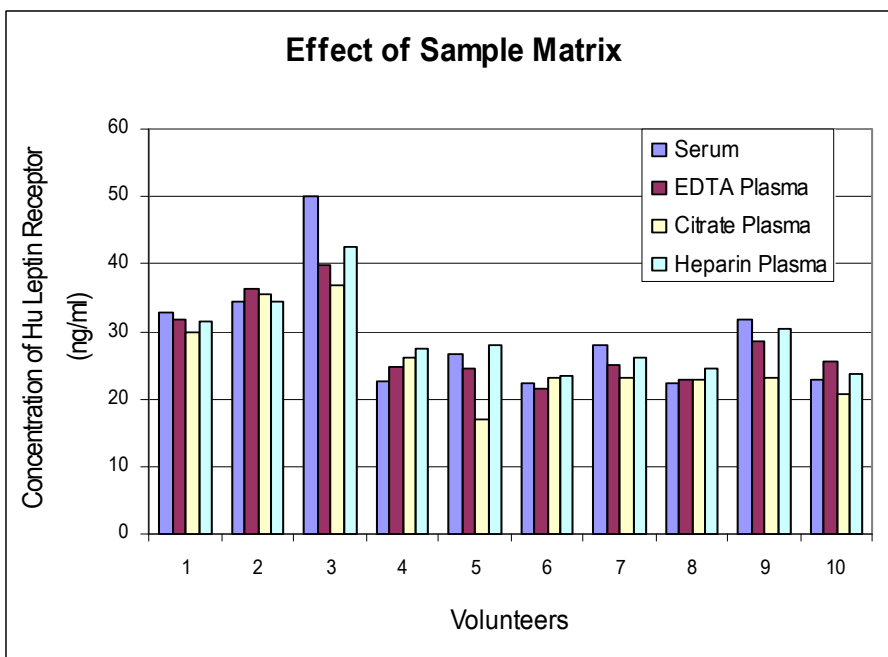


Figure 3: Leptin receptor levels measured using Human Leptin Receptor ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

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

Samples should be stored at -20°C. However, no decline in concentration of leptin receptor was observed in serum and plasma samples after 10 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	49.75	41.48	38.82	35.69
	2-8°C, 1 day	47.13	43.03	41.38	41.19
	2-8°C, 10 days	45.04	44.28	46.02	40.49
2	-20°C	22.36	23.27	21.70	22.97
	2-8°C, 1 day	21.79	24.54	21.81	24.10
	2-8°C, 10 days	23.56	22.69	22.93	19.35
3	-20°C	33.28	33.34	35.09	34.46
	2-8°C, 1 day	35.80	33.49	30.82	32.29
	2-8°C, 10 days	35.15	33.96	31.77	35.45

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human leptin receptor 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	21.39	20.89	16.77	21.79
	3x	18.69	18.31	16.20	22.72
	5x	20.06	20.47	16.43	21.15
2	1x	26.89	24.71	23.33	27.14
	3x	26.77	26.91	22.29	26.58
	5x	25.24	24.80	20.93	24.36
3	1x	18.07	18.51	16.00	18.96
	3x	17.57	17.95	17.19	19.73
	5x	19.39	18.55	17.29	19.62

- **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 receptor levels with the assay.

14. DEFINITION OF THE STANDARD

The standard used in this kit is recombinant fusion protein chimera, which is composed of human IgG-Fc-fragment and human OB-R and is different from the native soluble OB-R that is measured in human serum. As a result of glycosylation, recombinant human OB-R/Fc chimera migrates as a 155-175 kDa protein in SDS-PAGE.

15. METHOD COMPARISON

BioVendor Human Leptin Receptor 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 leptin receptor:

- Lahlou N. et al.: Soluble leptin receptor in serum of subjects with complete resistance to leptin: *Diabetes*; **19**:1347-1352 (2000)
- Auwerx J. and Staels B.: Leptin (Review article). *The Lancet*; **13**:737 (1998)
- Chen H. et al.: Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. *Cell*; **84**:491-495 (1996)
- Ciofii J. A. et al.: Novel B219/OB receptor isoforms: possible role of leptin in hematopoiesis and reproduction. *Nature Med.*; **2**:585-589 (1996)
- Houseknecht KL and Portocarrero CP: Leptin and its receptors: Regulators of whole body energy homeostasis. *Domestic animal endocrinology*; **15 (6)**:457-475 (1998)
- Lee GH et al.: Abnormal splicing of the leptin receptor in diabetic mice. *Nature*; **379**:632-635 (1996)
- Tartaglia LA et al.: Identification and Expression Cloning of a Leptin Receptor, OB-R. *Cell*; **83**:1263-1271 (1995)
- Tartaglia LA: The leptin receptor. *J. Biol. Chem.*; **272**:6093-6096 (1997)

»» References to this product:

- Haluzikova D, Dostalova I, Kavalkova P, Roubicek T, Mraz M, Papezova H, Haluzik M. Serum concentrations of adipocyte fatty acid binding protein in patients with anorexia nervosa. *Physiol Res*. 2009;58 (4):577-81
- Dostalova I, Kavalkova P, Haluzikova D, Lacinova Z, Mraz M, Papezova H, Haluzik M. Plasma concentrations of fibroblast growth factors 19 and 21 in patients with anorexia nervosa. *J Clin Endocrinol Metab*. (2008)
- Anderson PD, Mehta NN, Wolfe ML, Hinkle CC, Pruscino L, Comiskey LL, Tabita-Martinez J, Sellers KF, Rickels MR, Ahima RS, Reilly MP . Innate immunity modulates adipokines in humans. *J Clin Endocrinol Metab* . Jun;92(6):2272-9 (2007)
- Hoefle G, Saely CH, Risch L, Rein P, Koch L, Schmid F, Aczel S, Marte T, Langer P, Drexel H . Leptin, leptin soluble receptor and coronary atherosclerosis. *Eur J Clin Invest* . Aug;37(8):629-36 (2007)
- Proto C, Romualdi D, Cento RM, Romano C, Campagna G, Lanzzone A . Free and total leptin serum levels and soluble leptin receptors levels in two models of genetic obesity: the Prader-Willi and the Down syndromes. *Metabolism* . Aug;56(8):1076-80 (2007)
- Martos-Moreno GA, Barrios V, Argente J . Normative data for adiponectin, resistin, interleukin 6, and leptin/receptor ratio in a healthy Spanish pediatric population: relationship with sex steroids. *Eur J Endocrinol* . Sep;155(3):429-34 (2006)

- Paul G, Schaffler A, Neumeier M, Furst A, Bataille F, Buechler C, Muller-Ladner U, Scholmerich J, Rogler G, Herfarth H . Profiling adipocytokine secretion from creeping fat in Crohn's disease. *Inflamm Bowel Dis* . Jun;12(6):471-7 (2006)
- Presle N, Pottier P, Dumond H, Guillaume C, Lopicque F, Pallu S, Mainard D, Netter P, Terlain B . Differential distribution of adipokines between serum and synovial fluid in patients with osteoarthritis. Contribution of joint tissues to their articular production. *Osteoarthritis Cartilage* . Jul;14(7):690-5 (2006)
- Stejskal D, Svoboda I . Stanovení koncentrace solubilního leptinového receptoru (Ob-Re) pomocí nové metody ELISA. *Chem Listy* . 809-812 (2006)
- Dostalova I, Kopsky V, Duskova J, Papezova H, Pacak K, Nedvidkova J . Leptin concentrations in the abdominal subcutaneous adipose tissue of patients with anorexia nervosa assessed by in vivo microdialysis. *Regul Pept* . May 15;128(1):63-8 (2005)
- Housova J, Wilczek H, Haluzik MM, Kremen J, Krizova J, Haluzik M . Adipocyte-derived hormones in heroin addicts: the influence of methadone maintenance treatment. *Physiol Res* . 54(1):73-8 (2005)
- Jarkovska Z, Hodkova M, Sazamova M, Rosicka M, Dusilova-Sulkova S, Marek J, Justova V, Lacinova Z, Haluzik M, Haas T, Krsek M . Plasma levels of active and total ghrelin in renal failure: a relationship with GH/IGF-I axis. *Growth Horm IGF Res* . Dec;15(6):369-76 (2005)
- Papadopoulos DP, Makris TK, Krespi PG, Poulakou M, Paizis IA, Hatzizacharias AN, Perrea D, Votteas VV . Human soluble leptin receptor number in healthy normotensive individuals with high normal blood pressure. *Am J Hypertens* . Jul;18(7):1001-4 (2005)
- Popruk S, Tungtrongchitr R, Pongpaew P, Phonrat B, Tungtongchitr A, Tribunyatkul S, Paksanont S, Vudhivai N, Schelp FP . Relationship between soluble leptin receptor, leptin, lipid profiles and anthropometric parameters in overweight and obese Thai subjects. *J Med Assoc Thai* . Feb;88(2):220-7 (2005)
- Szalay F, Folhoffer A, Horvath A, Csak T, Speer G, Nagy Z, Lakatos P, Horvath C, Habior A, Tornai I, Lakatos PL . Serum leptin, soluble leptin receptor, free leptin index and bone mineral density in patients with primary biliary cirrhosis. *Eur J Gastroenterol Hepatol* . Sep;17(9):923-8 (2005)
- Verhaeghe J, van Bree R, Lambin S, Caluwaerts S . Adipokine profile and C-reactive protein in pregnancy: effects of glucose challenge response versus body mass index. *J Soc Gynecol Investig* . Jul;12(5):330-4 (2005)
- Zemanova M, Petruzalka L, Pazdrova G, Haluzik M, Novak F, Svobodnik A . Changes in leptin, leptin receptor and fatty acids levels during chemoradiotherapy for oesophageal cancer. *Cas Lek Cesk* . 144(12):811-6 (2005)
- Tsiotra PC, Pappa V, Koukourava A, Economopoulos T, Tsigos C, Raptis SA . Expression of leptin receptors in mononuclear cells from myelodysplastic syndromes and acute myeloid leukemias. *Acta Haematol* . 114(2):71-7 (2005)
- Housova J, Anderlova K, Krizova J, Haluzikova D, Kremen J, Kumstyrova T, Papezova H, Haluzik M . Serum adiponectin and resistin concentrations in patients with restrictive and binge/purge form of anorexia nervosa and bulimia nervosa. *J Clin Endocrinol Metab* . Dec 14 (2004)







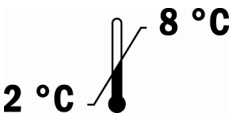

- Chatzantoni K, Papathanassopoulos P, Gourzoulidou E, Mouzaki A . Leptin and its soluble receptor in plasma of patients suffering from remitting-relapsing multiple sclerosis (MS) In vitro effects of leptin on type-1 and type-2 cytokine secretion by peripheral blood mononuclear cells, T-cells and monocytes of MS patients. *J Autoimmun* . Sep;23(2):169-77 (2004)
- Krsek M, Silha JV, Jezkova J, Hana V, Marek J, Weiss V, Stepan JJ, Murphy LJ . Adipokine levels in Cushing's syndrome; elevated resistin levels in female patients with Cushing's syndrome. *Clin Endocrinol (Oxf)* . Mar;60(3):350-7 (2004)
- Meier U, Gressner AM . Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. *Clin Chem* . Sep;50(9):1511-25 (2004)
- Nuamah MA, Yura SA, Sagawa NA, Itoh H, Miese H, Korita D, Kakui K, Takemura M, Ogawa Y, Nakao K, Fujii S . Significant increase in maternal plasma leptin concentration in induced delivery: a possible contribution of pro-inflammatory cytokines to placental leptin secretion. *Endocr J* . Apr;51(2):177-87 (2004)
- Wolfe BE, Jimerson DC, Orlova C, Mantzoros CS . Effect of dieting on plasma leptin, soluble leptin receptor, adiponectin and resistin levels in healthy volunteers. *Clin Endocrinol (Oxf)* . Sep;61(3):332-8 (2004)
- Buyan N, Ozkaya O, Bideci A, Soylemezoglu O, Cinaz P, Gonen S, Kalman S, Bakkaloglu S, Hasanoglu E. Leptin, soluble leptin receptor, and transforming growth factor-beta1 levels in minimal change nephrotic syndrome. *Pediatr Nephrol*. Jul;29(8) (2003)
- Gavrilu A, Peng CK, Chan JL, Mietus JE, Goldberger AL, Mantzoros CS. Diurnal and ultradian dynamics of serum adiponectin in healthy men: comparison with leptin, circulating soluble leptin receptor, and cortisol patterns. *J Clin Endocrinol Metab* . Jun;88(6):2838-43 (2003)
- Kalousova M, Sulkova S, Zima T, Deppisch R, Beck W, Bednarova V, Fortova M, Tesar V . Advanced glycation end products in hemodialyzed patients with diabetes mellitus correlate with leptin and leptin/body fat ratio. *Ren Fail* . Mar;25(2):277-86 (2003)
- Kado N, Kitawaki J, Koshihara H, Ishihara H, Kitaoka Y, Teramoto M, Honjo H . Relationships between the serum levels of soluble leptin receptor and free and bound leptin in non-pregnant women of reproductive age and women undergoing controlled ovarian hyperstimulation. *Hum Reprod* . Apr;18(4):715-20 (2003)
- Kalman S, Buyan N, Bayazit AK, Bideci A, Bakkaloglu S, ozkaya O, Soylemezoglu O. The role of leptin and soluble leptin receptor on the nutritional parameters in pediatric CPD patients. *Nephrology Dialysis Transplantation* . Volume 18 Supplement 4 June 2003
- Krizova J, Sulkova S, Bednarova V, Parizkova J, Kotrlíkova E, Haluzik M . Soluble leptin receptor levels in patients with chronic renal failure. *Physiol Res* . 52(3):347-351 (2003)
- Lenchik L, Register TC, Hsu FC, Lohman K, Nicklas BJ, Freedman BI, Langefeld CD, Carr JJ, Bowden DW. Adiponectin as a novel determinant of bone mineral density and visceral fat. *Bone* . Oct;33(4):646-51 (2003)
- Nuamah MA, Sagawa N, Yura S, Mise H, Itoh H, Ogawa Y, Nakao K, Fujii S . Free-to-total leptin ratio in maternal plasma is constant throughout human pregnancy. *Endocr J* . Aug;50(4):421-8 (2003) Welt CK, Schneyer AL, Heist K, Mantzoros CS . Leptin and soluble leptin receptor in follicular fluid. *J Assist Reprod Genet* . Dec;20(12):495-501 (2003)

- Rosicka M, Krsek M, Matoulek M, Jarkovska Z, Marek J, Justova V V, Lacinova Z . Serum ghrelin levels in obese patients: the relationship to serum leptin levels and soluble leptin receptors levels. *Physiol Res* . Feb;52(1):61-66 (2003)
- Sandhofer A, Laimer M, Ebenbichler CF, Kaser S, Paulweber B, Patsch JR. Soluble leptin receptor and soluble receptor-bound fraction of leptin in the metabolic syndrome. *Obes Res* . Jun;11(6):760-8 (2003)
- Stejskal D, Ruzicka V, Adamovska S, Jurak Mann DR, Johnson AO, Gimpel T, Castracane VD . Changes in circulating leptin, leptin receptor, and gonadal hormones from infancy until advanced age in humans. *J Clin Endocrinol Metab* Jul . Jul;88(7):3339-45 (2003)
- Teplan V, Schuck O, Stolova M, Vitko S . Obesity and hyperhomocysteinaemia after kidney transplantation. *Nephrol Dial Transplant* . Jul;18 Suppl 5:v71-3 (2003)
- Yiannakouris N, Melistas L, Yannakoulia M, Mungal K, Mantzoros CS . The -2548G/A polymorphism in the human leptin gene promoter region is associated with plasma free leptin levels; interaction with adiposity and gender in healthy subjects. *Hormones* . 2(4):229-236 (2003)
- Yannakoulia M, Yiannakouris N, Bluher S, Matalas AL, Klimis-Zacas D, Mantzoros CS. Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. *J Clin Endocrinol Metab* . Apr;88(4):1730-6 (2003)
- Chan JL, Blüher S, Yiannakouris N, Suchard MA, Kratzsch J, Mantzoros CS . Regulation of Circulating Soluble Leptin Receptor Levels By Gender, Adiposity, Sex Steroids, and Leptin. Observational and Interventional Studies in Humans. *Diabetes* . 51(7):2105-2112 (2002)
- Haluzik M, Markova M, Slaby JJ, Jiskra J, Krizova J, Hass T . The changes of serum leptin and soluble leptin receptor levels in patients undergoing mobilization of peripheral blood stem cells before autologous stem cells transplantation. *Endocr Res* . Aug;28(3):189-97 (2002)
- Krsek M, Rosicka M, Haluzik M, Svobodova J, Kotrlíkova E, Justova V, Lacinova Z, Jarkovska Z . Plasma ghrelin levels in patients with short bowel syndrome. *Endocr Res* . Feb-May;28(1-2):27-33 (2002)
- Lahlou N, Issad T, Lebouc Y, Carel JC, Camoin L, Roger M, Girard J. Mutations in the human leptin and leptin receptor genes as models of serum leptin receptor regulation. *Diabetes* . 51(6):1980-5 (2002)
- Laimer M, Ebenbichler CF, Kaser S, Sandhofer A, Weiss A, Nehoda H, Aigner F, Patsch JR . Weight Loss Increases Soluble Leptin Receptor Levels and the Soluble Receptor Bound Fraction of Leptin. *Obesity Research* . 10(7):597-601 (2002)
- Manzella D, Parillo M, Razzino T, Gnasso P, Buonanno S, Gargiulo A, Caputi M, Paolisso G . Soluble leptin receptor and insulin resistance as determinant of sleep apnea. *Int J Obes Relat Metab Disord* . 26(3):370-5 (2002)
- Monteleone P, Fabrazzo M, Tortorella A, Fuschino A, Maj M. Opposite modifications in circulating leptin and soluble leptin receptor across the eating disorder spectrum. *Mol Psychiatry* . 7(6):641-6 (2002)
- Ogier V, Ziegler O, Mejean L, Nicolas JP, Stricker-Krongrad A. Obesity is associated with decreasing levels of the circulating soluble leptin receptor in humans. *Int J Obesity* . 26(4):496-503 (2002)

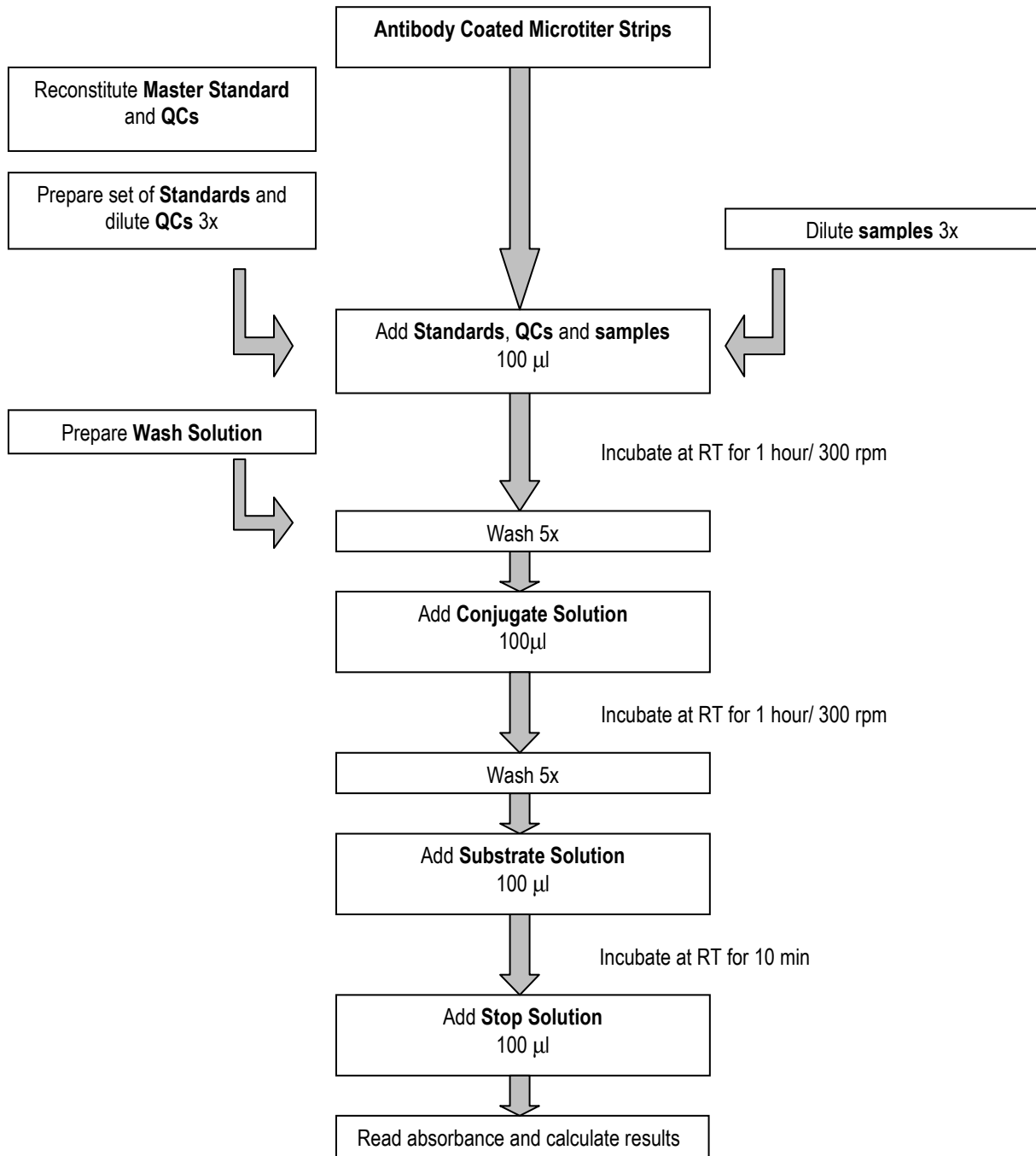
- Pecoits-Filho R, Nordfors L, Heimbürger O, Lindholm B, Anderstam B, Marchlewska A, Stenvinkel P. Soluble leptin receptors and serum leptin in end-stage renal disease: relationship with inflammation and body composition. *Eur J Clin Invest Nov* . 32(11):811-7 (2002)
- Shimizu H, Shimomura K, Negishi M, Masunaga M, Uehara Y, Sato N, Shimomura Y, Kasai K, Mori M . Circulating concentrations of soluble leptin receptor: influence of menstrual cycle and diet therapy. *Nutrition* . 18(4):309-12 (2002)
- van Dielen FM, van 't Veer C, Buurman WA, Greve JW . Leptin and soluble leptin receptor levels in obese and weight-losing individuals. *J Clin Endocrinol Metab* . 87(4):1708-16 (2002)
- Malincikova J, Stejskal D, Hrebicek J . Serum leptin and leptin receptors in healthy prepubertal children: relations to insulin resistance and lipid parameters, body mass index (BMI), tumor necrosis factor alpha (TNF alpha), heart fatty acid binding protein (hFABP), and IgG anticardiolipin. *Acta Univ Palacki Olomuc Fac Med* . 143:51-7 (2000)
- Stejskal D, Bartek J, Ruzicka V, Stejskal P, Jedelsky L, Oral I, Horalik D, Zurek M . Determination of leptin receptor in the serum and relations to laboratory and anthropological parameters in patients with atherosclerotic complications. *Acta Univ Palacki Olomouc Fac Med* . 142:89-92 (1999)

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

18. EXPLANATION OF SYMBOLS

	Catalogue number
	Content
	Lot number
	See instructions for use
	Biological hazard
	Expiry date
	Storage conditions
	Identification of packaging materials

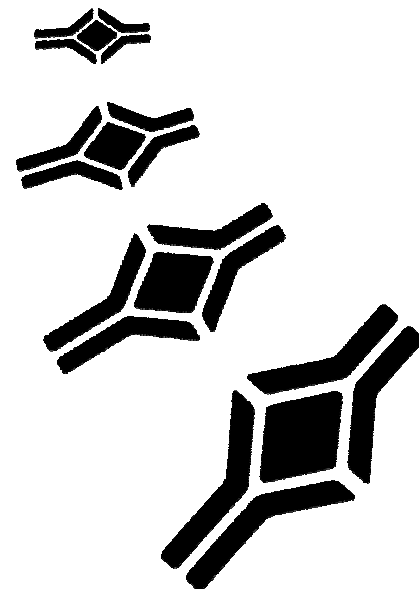
Assay Procedure Summary



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

NOTES





HEADQUARTERS: BioVendor - Laboratorni medicina 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
EUROPEAN UNION: BioVendor GmbH	Im Neuenheimer Feld 583	D-69120 Heidelberg GERMANY	Phone: +49-6221-433-9100 Fax: +49-6221-433-9111	E-mail: infoEU@biovendor.com
USA, CANADA AND MEXICO: BioVendor LLC	128 Bingham Rd. Suite 1300	Asheville, NC 28806 USA	Phone: +1-828-575-9250 +1-800-404-7807 Fax: +1-828-575-9251	E-mail: infoUSA@biovendor.com
CHINA - Hong Kong Office: BioVendor Laboratories Ltd	Room 4008 Hong Kong Plaza, No.188	Connaught Road West Hong Kong, CHINA	Phone: +852-2803-0523 Fax: +852-2803-0525	E-mail: infoHK@biovendor.com
CHINA – Mainland Office: BioVendor Laboratories Ltd	Room 2917, 29/F R & F Ying Feng Plaza, No.2 Huaqiang road	Pearl River New Town Guang Zhou, CHINA	Phone: +86-20-38065519 Fax: +86-20-38065529	E-mail: infoCN@biovendor.com