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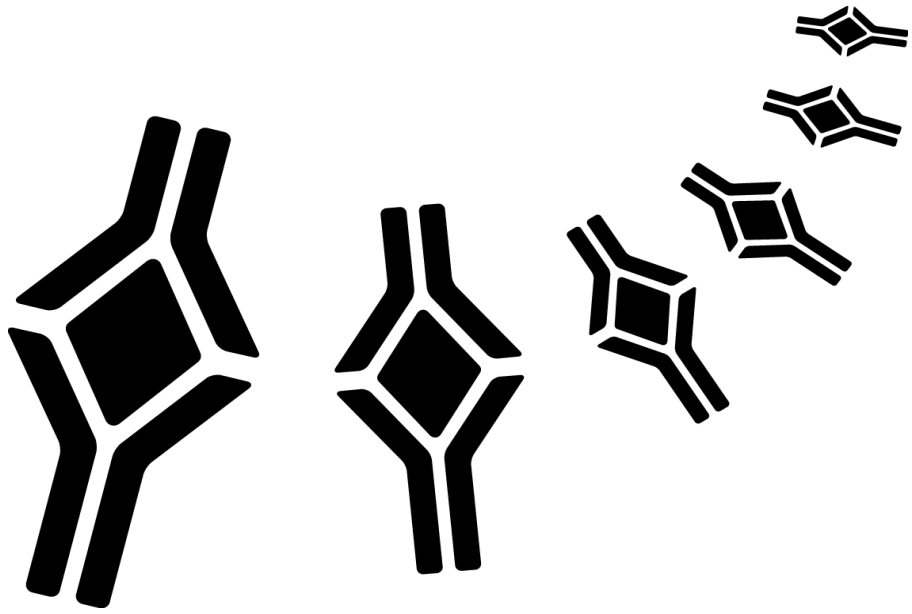
Research
and Diagnostic Products



HUMAN CHEMERIN ELISA

Product Data Sheet

Cat. No.: RD191136200R

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 RD191136200R Human Chemerin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human chemerin.

Features

- **It is intended for research use only**
- The total assay time is less than 3.5 hours
- The kit measures chemerin in serum and plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- Standard is recombinant protein based
- Quality Controls are human serum based. No animal sera are used
- 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

Chemerin, also known as tazarotene induced gene 2 (TIG2) and retinoic acid receptor responder 2 (RERRES2) is a novel chemoattractant protein secreted as an 18-kDa inactive pro-protein. Prochemerin undergoes extracellular serine protease cleavage of the C-terminal portion of the protein to generate the 16-kDa active chemerin, which is present in plasma, serum and hemofiltrate. Active chemerin is abundant in ascitic fluid from ovarian cancer patients and synovial fluid from patients with arthritis.

Signaling by chemerin is mediated by the seven-transmembrane-spanning G protein-coupled receptor called chemokine like receptor-1 (CMKLR1, ChemR23) or chemerinR. Both chemerin and chemerinR mRNA expression dramatically increased during the differentiation of preadipocytes into adipocytes. Chemerin induced the phosphorylation of extracellular signal – regulated kinase 1/2 (ERK 1/2) and lipolysis in differentiated adipocytes and 3T3-L1 cells, stimulated intracellular calcium release and inhibited cAMP accumulation. Chemerin disruption has some effect on adipogenesis in vitro, but regulation of gene expression and lipolysis in mature adipocytes suggests a wider role in lipid and carbohydrate metabolisms, and perhaps insulin sensitivity. Local production of chemerin regulates adipogenesis and through its receptor or possible other receptor can modulate a variety of functions in mature adipocytes. Adipocytes purified from adipose tissue contain high levels of chemerin mRNA; however, substantial expression in stromal vascular cells suggest that production in nonadipocytes may also be important.

Recent findings strongly suggest that white adipose tissue serves as both a primary source of chemerin secretion as well as a target for autocrine/paracrine chemerin signalling. A critical function of chemerin/chemerinR is to regulate adipogenesis and metabolic homeostasis in adipocytes in mice and humans. Expression of chemerin in white adipose tissue is much higher than in brown adipose tissue in lean subjects. Chemerin and chemerinR knockdowns largely abrogate adipocyte differentiation. Thus, chemerin is essential in early differentiation processes and may contribute or regulate critical early events in adipogenesis. Results also indicate that chemerin and chemerinR could have an important biological role in the formation of white adipose tissue during normal or in pathological states.

Another study showed that circulating levels of chemerin correlated with body mass index, plasma triacylglycerol concentrations, and blood pressure but were not altered by the presence of type 2 diabetes mellitus. Its relationship with body mass index and aspects of metabolic syndrome suggests a larger role for this protein in obesity-associated complications.

<u>Areas of investigation:</u>
Metabolic syndrome
Obesity
Glucose and lipid homeostase

4. TEST PRINCIPLE

In the Biovendor Human Chemerin ELISA, standards, quality controls and samples are incubated in microtitration wells pre-coated with polyclonal anti-human chemerin antibody. After a 60 minute incubation followed by washing, biotin labelled polyclonal anti-human chemerin antibody is added and incubated with the captured chemerin 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 chemerin. A standard curve is constructed by plotting absorbance values against chemerin concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

5. PRECAUTIONS

- **For professional use only**
- Wear gloves and laboratory coats when handling immunodiagnostic materials
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary
- The materials must not be pipetted by mouth

6. TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware
- Use deionized (distilled) water, stored in clean containers
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

7. REAGENT SUPPLIED

Kit Components	*State*	*Quantity*
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody Conc. (40x)	concentrated	0.35 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
Dilution Buffer	ready to use	50 ml
Biotin-Ab Diluent	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 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 550-650 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.

Streptavidin-HRP Conjugate
Dilution Buffer
Biotin-Ab Diluent
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 Chemerin 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 human chemerin in the stock solution is **8 ng/ml.**

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	8 ng/ml
300 µl of stock	300 µl	4 ng/ml
300 µl of 4 ng/ml	300 µl	2 ng/ml
300 µl of 2 ng/ml	300 µl	1 ng/ml
300 µl of 1 ng/ml	300 µl	0.5 ng/ml
300 µl of 0.5 ng/ml	300 µl	0.25 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.

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 the kit works in accordance with PDS and CoA and that ELISA test was carried out properly.

Biotin Labelled Antibody Conc. (40x)
Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (40x) with 39 parts Biotin-Ab Diluent. Example: 25 µl of Biotin Labelled Antibody Concentrate (40x) + 975 µl of Biotin-Ab Diluent for 1 strip (8 wells).
Stability and storage:
Opened Biotin Labelled Antibody Concentrate (40x) 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 human chemerin in serum and plasma (EDTA, citrate, heparin) samples.

Samples should be assayed immediately after collection or should be stored at -20°C or -70°C. Thoroughly mix 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 just prior to performing the assay 100x with Dilution Buffer, e.g. 5 µl of sample + 495 µl of Dilution Buffer for singlets or duplicates. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:
Serum samples should be stored at -20°C, or preferably at -70°C for long-term storage.
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 chemerin.

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 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 **15 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.
12. Stop the colour development by adding **100 µl** of Stop Solution.
13. Determine the absorbance of each well on 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 chemerin 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 8**	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 4**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 2**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 1**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 0.5**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 0.25**	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 Standards curve is constructed by plotting the 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 chemerin (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. 2 ng/ml (from standard curve) x 100 (dilution factor) = 200 ng/ml.

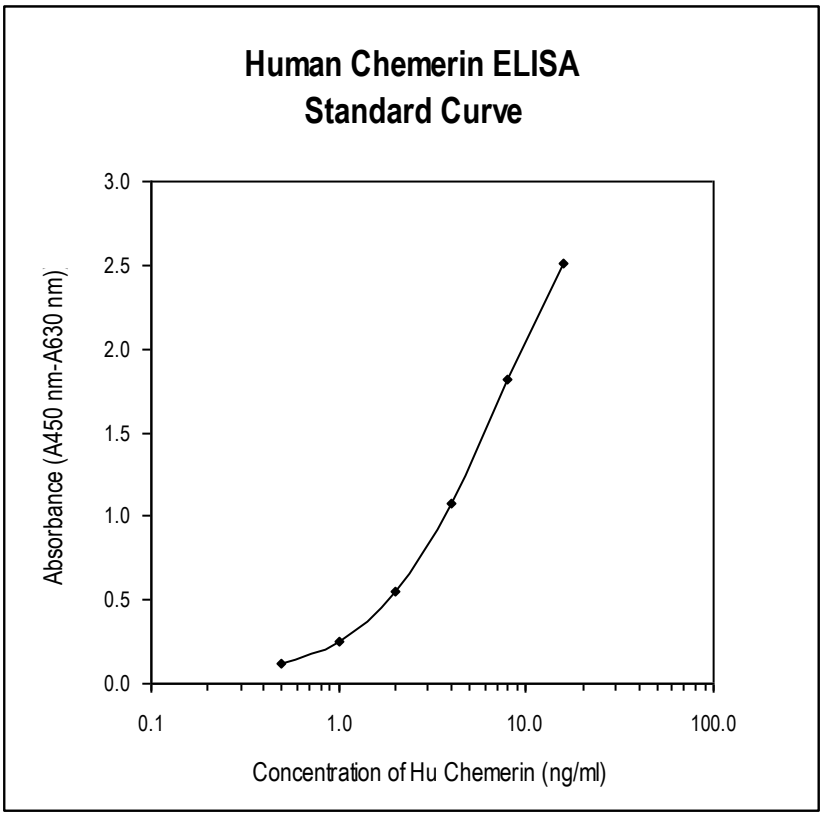


Figure 2: Typical Standard Curve for Human Chemerin ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Chemerin ELISA are presented in this chapter

- **Sensitivity**

Limit of detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A_{blank} + $3xSD_{blank}$) is calculated from the real human chemerin values in wells and is: 0.1 ng/ml.
* Dilution Buffer is pipetted into Blank wells.

- **Limit of assay**

Results exceeding human chemerin level of 8 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the chemerin concentration.

- **Specificity**

The antibodies used in this ELISA are specific for human chemerin.

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

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

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	177.5	9.0	5.1
2	263.6	18.4	7.0

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	140.6	11.6	8.3
2	222.7	15.5	6.9

- **Spiking Recovery**

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

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	211.0	-	-
	416.5	411.0	101.3
	316.0	311.0	101.6
	247.5	261.0	94.8
2	182.0	-	-
	354.0	382.0	92.7
	282.0	282.0	100.0
	204.5	232.0	88.1

- **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	-	335.0	-	-
	2x	170.0	167.5	101.5
	4x	85.0	83.8	101.5
	8x	42.0	41.9	100.3
2	-	225.0	-	-
	2x	115.0	112.5	102.2
	4x	60.0	56.3	106.7
	8x	30.0	28.1	106.7

- **Effect of sample matrix**

EDTA, citrate and heparin plasmas were compared to respective serum samples from the same 10 individuals.
Results are shown below:

Volunteer No.	*Serum (ng/ml)*	*Plasma (ng/ml)*		
		EDTA	*Citrate*	*Heparin*
1	175.0	210.0	180.0	200.0
2	155.0	240.0	165.0	285.0
3	205.0	255.0	245.0	220.0
4	190.0	215.0	170.0	260.0
5	190.0	170.0	125.0	195.0
6	180.0	125.0	115.0	230.0
7	240.0	180.0	155.0	230.0
8	260.5	307.0	241.0	267.0
9	199.0	176.5	160.0	212.5
10	302.5	285.0	231.0	252.5
Mean (ng/ml)	**202.5**	**212.4**	**175.6**	**235.9**
Mean Plasma/Serum (%)	**-**	**105**	**87**	**116**

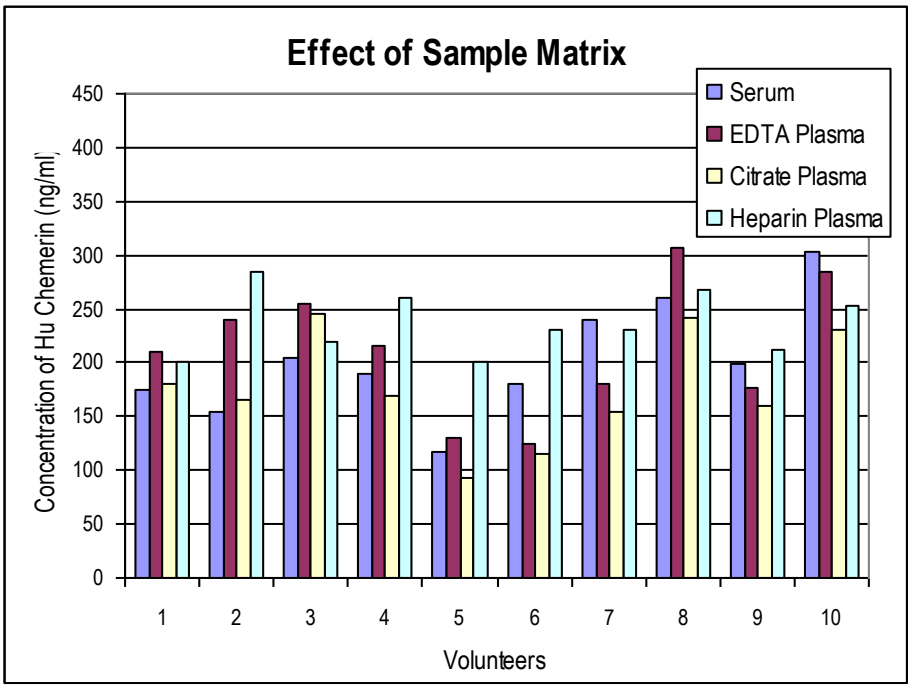


Figure 3: Chemerin levels measured using Human Chemerin ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

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

Samples should be stored at -20°C. However, no significant decline in concentration of human chemerin 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	210.0	230.0	180.0	215.0
	2-8°C, 1 day	240.0	255.0	180.0	240.0
	2-8°C, 7 days	230.0	240.0	210.0	235.0
2	-20°C	270.0	260.0	235.0	225.0
	2-8°C, 1 day	280.0	255.0	225.0	235.0
	2-8°C, 7 days	215.0	260.0	200.0	210.0
3	-20°C	330.0	240.0	160.0	235.0
	2-8°C, 1 day	320.0	215.0	135.0	230.0
	2-8°C, 7 days	325.0	205.0	122.5	230.0

- **Effect of Freezing/Thawing**

No significant decline was observed in concentration of human chemerin in serum 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	215.0	235.0	195.0	245.0
	3x	200.0	245.0	185.0	330.0
	5x	220.0	160.0	205.0	295.0
2	1x	155.0	185.0	140.0	205.0
	3x	160.0	180.0	160.0	195.0
	5x	160.0	170.0	140.0	145.0
3	1x	215.0	253.0	190.0	193.0
	3x	210.0	200.0	160.0	255.0
	5x	235.0	198.0	115.0	150.0

14. DEFINITION OF THE STANDARD

The recombinant human chemerin is used as the Standard. The recombinant human chemerin (Glu 21 – Ser 157), produced in *E.coli*, is 16 kDa protein containing 137 amino acid residues of the human chemerin and methionyl 138.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 130 unselected donors (64 men + 66 women) 3-88 years old were assayed with the Biovendor Human Chemerin ELISA in our laboratory.

- **Age and Sex dependent distribution of chemerin**

Sex	*Age (years)*	*n*	*Mean*	*SD*	*Min.*	*Max.*
			Chemerin (ng/ml)			
Men	3-19	5	176.3	42.8	136.5	248.0
	20-39	8	178.1	26.7	133.5	229.5
	40-59	24	186.8	36.9	117.5	270.0
	60-79	21	206.0	43.2	132.0	288.5
	80-88	6	234.3	39.1	169.0	285.0
Women	3-19	6	207.2	28.3	155.0	240.0
	20-39	14	199.3	29.6	140.0	245.0
	40-59	24	206.4	42.2	142.0	313.5
	60-79	20	247.5	63.1	170.0	452.5
	80-86	2	266.5	37.0	229.5	303.5

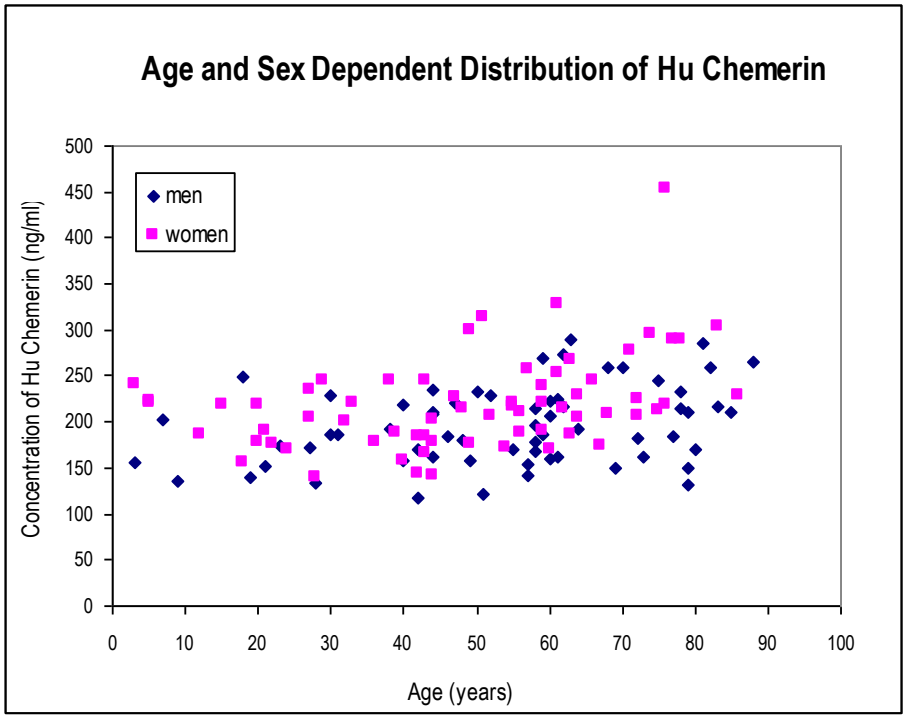


Figure 4: Human chemerin concentration plotted against donor age and sex.

- **Reference range**

The data quoted in these instructions should be used for guidance only. 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 references ranges for chemerin levels with the assay.

16. METHOD COMPARISON

The Biovendor Human Chemerin ELISA was compared to another commercial ELISA immunoassay, by measuring 40 serum samples. The following correlation graph was obtained.

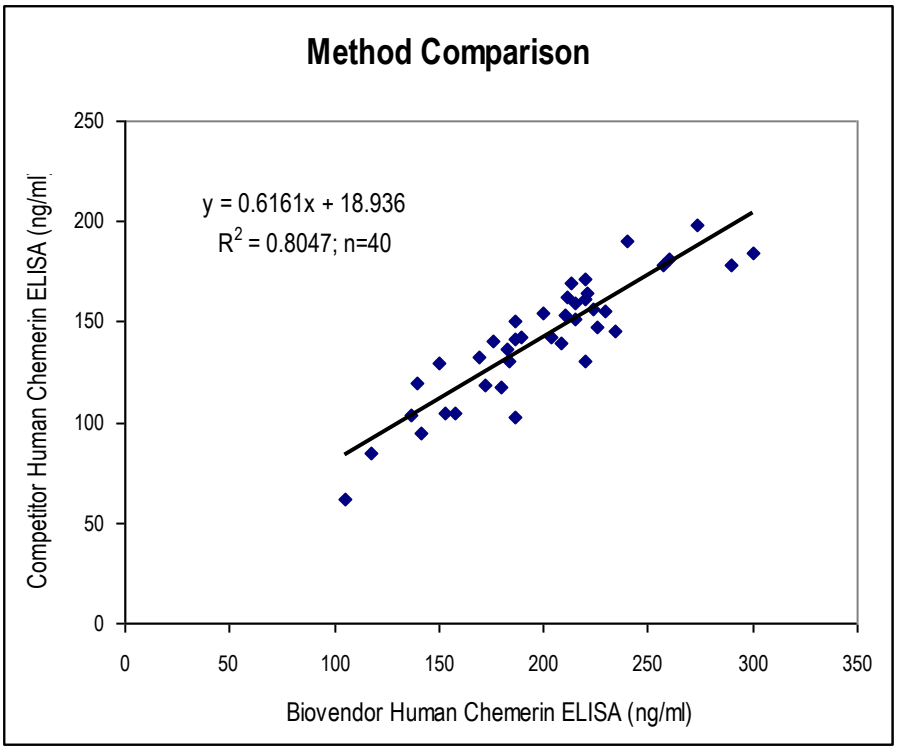


Figure 5: Method comparison.

17. 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

18. REFERENCES

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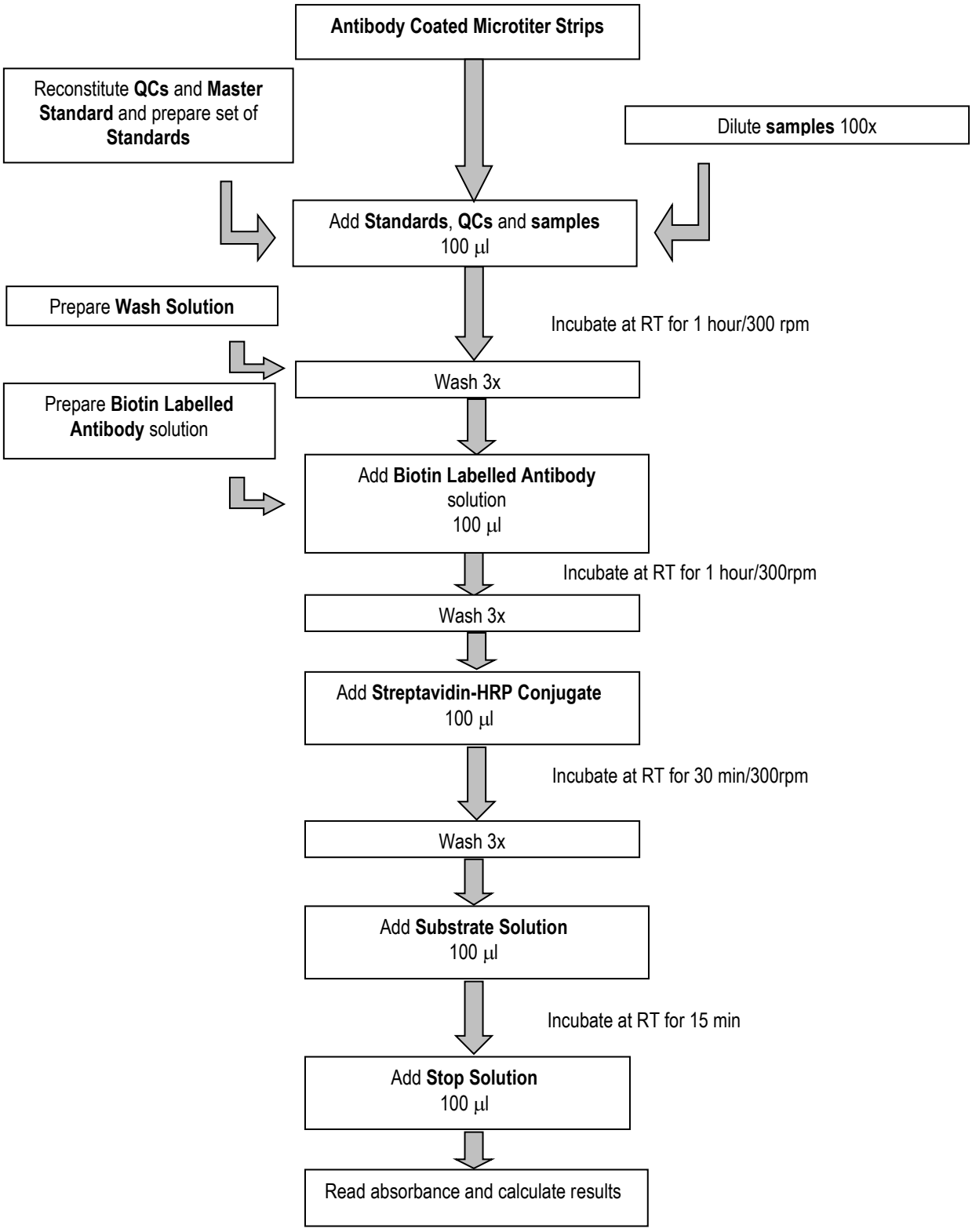
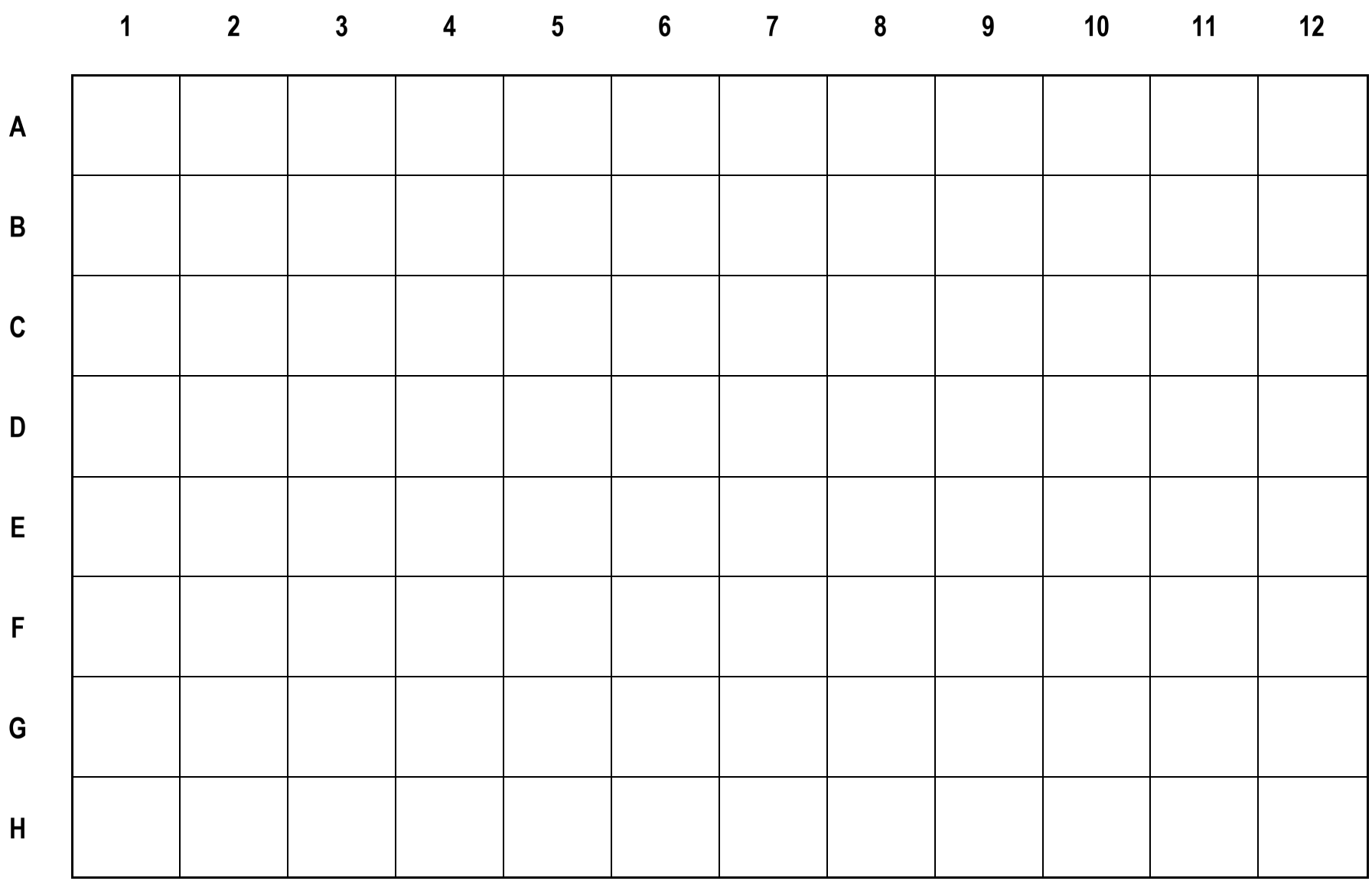
For more references on this product see our WebPages at www.biovendor.com

19. EXPLANATION OF SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	Attention, see instructions for use
	Potential biological hazard
	Expiry date
	Storage conditions
	Name and registered office of the manufacturer

Assay Procedure Summary

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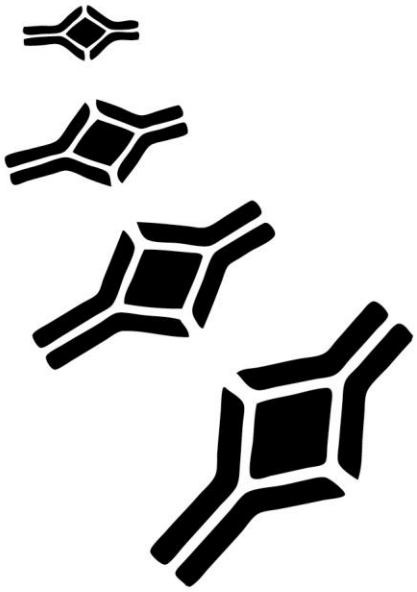

BioVendor – Laboratorní medicína a.s.
Karasek 1767/1, 621 00 Brno, Czech Republic
Phone: +420-549-124-185, Fax: +420-549-211-460
E-mail: info@biovendor.com, sales@biovendor.com
Web: www.biovendor.com

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