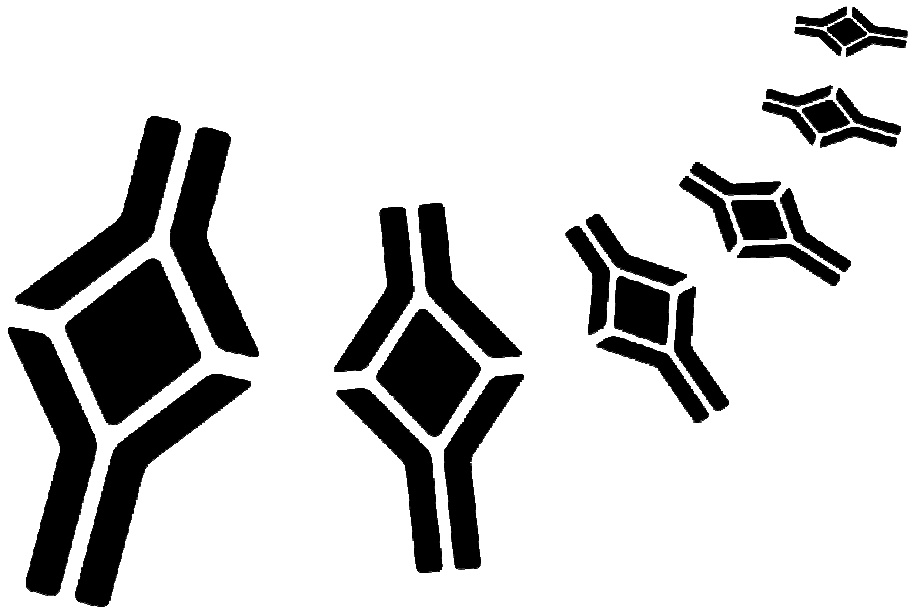
BioVendor
Research
and Diagnostic Products



HUMAN ANGIOPOIETIN-LIKE PROTEIN 4 ELISA

Product Data Sheet

Cat. No.: RD191073200R

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 RD191073200R Human Angiopoietin-like Protein 4 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human Angiopoietin-like Protein 4 (Angptl4).

Features

- **It is intended for research use only**
- The total assay time is less than 5 hours
- The kit measures Angptl4 in human serum, citrate and EDTA plasma
- 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

Angiopoietin-like protein 4 (Angptl4), initially known as hepatic fibrinogen/angiopoietin- related protein (HFARP), peroxisome proliferator-activated receptor-c (PPARc) angiopoietin-related gene (PGAR), or fasting-induced adipose factor (FIAF), is a secreted glycoprotein which structurally belongs to the angiopoietin/Angptl family. Human Angptl4 consists of 406 amino acids with a signal peptide directing secretion, an amino-terminal coiled-coil domain, a linker, and a carboxy-terminal fibrinogen-like domain.

Angptl4 is ubiquitously expressed in human tissues. The highest expression levels in humans are found in liver, followed by the small intestine, adipose tissue and heart. Angptl4 is strongly induced by fasting in white adipose tissue and liver and is an apoptosis survival factor for vascular endothelial cells under hypoxic conditions. The most studied function of Angptl4 is its role in the regulation of lipid metabolism, particularly as an inhibitor of lipoprotein lipase activity. Angptl4 has been implicated in the development of hypertriglyceridemia. It has been proposed that Angptl4 inhibits LPL activity in adipose tissue acting to reroute fatty acids away from fat to muscle and other tissues during fasting.

In patients with type 2 diabetes, serum levels of Angptl4 were significantly lower than those in healthy subjects, suggesting that decreased Angptl4 could be a causative factor of this disease. These results collectively indicate that Angptl4 exerts distinct effects on glucose and lipid metabolism, and that its beneficial effect on glucose homeostasis might be useful for the treatment of diabetes.

Angptl4 is involved in growth regulation of tumor mass and growth and differentiation of tumor cells. The action of this protein in published studies on tumor tissue have shown contradictory results. Some show Angptl4 as preventive of metastatic activity. This effect is caused by reduction of vascular (blood vessel) permeability. Another recently-published study, however, takes the opposite view. They describe Angptl4 as a factor causing increased vascular permeability and thus supporting the metastatic process in both the case of gastric cancer, esophageal and colorectal cancer.

<u>Areas of investigation:</u>
Energy metabolism and body weight regulation
Lipid metabolism
Glucose metabolism
Angiogenesis
Cell differentiation

4. TEST PRINCIPLE

In the BioVendor Human Angiopoietin-like Protein 4 ELISA, standards, quality controls and samples are incubated in microtitration wells pre-coated with polyclonal anti-human Angptl4 antibody. After 120 minutes incubation followed by washing, biotin labelled polyclonal anti-human Angptl4 antibody is added and incubated with the captured Angptl4 for 120 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 Angptl4. A standard curve is constructed by plotting absorbance values against Angptl4 concentrations of Standards and concentrations of unknown samples are determined using this standard curve.

5. PRECAUTIONS

- **For professional use only**
- Wear gloves and laboratory coats when handling immunodiagnostic materials
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- This kit contains components of animal origin. These materials 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	lyophilized	1 vial
Streptavidin-HRP Conjugate Conc. (100x)	concentrated	0.13 ml
Master Standard	lyophilized	2 vials
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
Dilution Buffer	ready to use	2 x 22 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.

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 Angptl4 Master Standard:
IMPORTANT: Refer to the Quality Control Data Sheet 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 Angptl4 in the stock solution is **60 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	-	60 ng/ml
250 µl of stock	250 µl	30 ng/ml
250 µl of 30 ng/ml	250 µl	15 ng/ml
250 µl of 15 ng/ml	250 µl	7.5 ng/ml
250 µl of 7.5 ng/ml	250 µl	3.75 ng/ml
250 µl of 3.75 ng/ml	250 µl	1.88 ng/ml
250 µl of 1.88 ng/ml	250 µl	0.94 ng/ml

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

Stability and storage:

Do not store the diluted Standard solutions.

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:

IMPORTANT: Refer to the Quality Control Data Sheet for current volume of Dilution Buffer needed for reconstitution of Biotin Labelled Antibody!!!

Reconstitute the lyophilized Biotin Labelled Antibody with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). Dilute reconstituted Biotin Labelled Antibody Concentrate (100x) with Dilution Buffer (e.g. 60 µl of Biotin Labelled Antibody Concentrate + 5 940 µl of Dilution Buffer for 6 strips (48 wells).

Stability and storage:

Biotin Labelled Antibody Concentrate (100x) is stable 1 month when stored at 2-8°C.

Do not store the diluted Biotin Labelled Antibody solution.

Streptavidin-HRP Conjugate Conc. (100x):
Prepare the working Streptavidin-HRP Conjugate solution by adding 1 part Streptavidin-HRP Conjugate Concentrate (100x) with 99 parts Dilution Buffer. Example: 60 µl of Streptavidin-HRP Concentrate (100x) + 5 940 µl of Dilution Buffer for 6 strips (48 wells).
Stability and storage:
Opened Streptavidin-HRP Conjugate Conc. (100x) is stable 3 months when stored at 2-8°C.
Do not store the diluted Streptavidin-HRP Conjugate 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 Angptl4 in serum, citrate and EDTA plasma.

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.

Preparation of samples:
Dilute samples 5x with Dilution Buffer just prior to the assay (e.g. 30 µl of sample + 120 µl of Dilution Buffer for singlets, or preferably 60 µl of sample + 240 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 human Angptl4.

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 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 **2 hours**, 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 **2 hours**, 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 Solution into each well.
8. Incubate the plate at room temperature (ca. 25°C) for **30 min**, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells **3-times** with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
10. Add **100 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
11. Incubate the plate for **10 minutes** 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 using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 - 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm. **The absorbance should be read within 5 minutes following step 12.**

Important: Do not to exceed the incubation temperature of 27°C, higher temperatures can result an increase in absorbance of standards and a decrease in concentration in tested samples.

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 Angptl4 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 60**	**QC HIGH**	Sample 7	Sample 15	Sample 23	Sample 31
B	**Standard 30**	**QC LOW**	Sample 8	Sample 16	Sample 24	Sample 32
C	**Standard 15**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
D	**Standard 7.5**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
E	**Standard 3.75**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
F	**Standard 1.88**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
G	**Standard 0.94**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
H	**Blank**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38

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, using the four-parameter algorithm. Results are reported as concentration of Angptl4 (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. 10 ng/ml (from standard curve) x 5 (dilution factor) = 50 ng/ml.

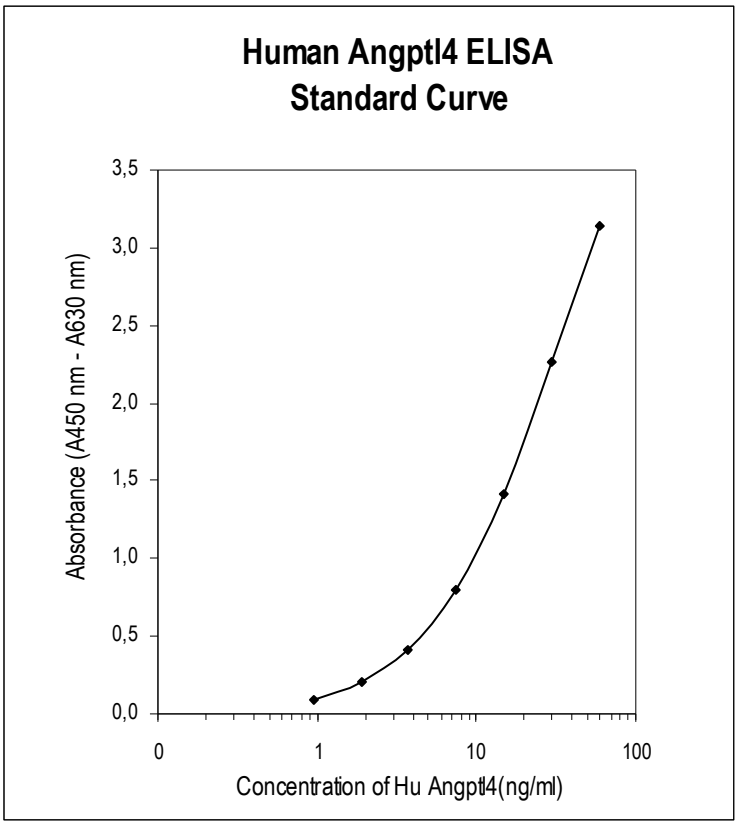


Figure 2: Typical Standard Curve for Human Angiopoietin-like Protein 4 ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Angiopoietin-like Protein 4 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 Angptl4 values in wells and is: 0.173 ng/ml.
* Dilution Buffer is pipetted into Blank wells.

- **Limit of Assay**

Results exceeding human Angptl4 level of 60 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the Angptl4 concentration.

- **Specificity**

The antibodies used in this ELISA are specific for human Angptl4 with no detectable crossreactivities to human Angptl3.

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	yes
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	107.3	3.8	3.6
2	24.9	1.0	4.1

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	35.0	2.3	6.7
2	131.4	6.8	5.2

- **Spiking Recovery**

Serum samples were spiked with different amounts of human Angptl4 and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	40.7	-	-
	100.6	115.7	86.9
	71.0	78.2	90.8
	60.3	59.5	101.4
2	41.9	-	-
	99.7	116.9	85.3
	75.0	79.4	94.5
	58.0	60.7	95.6

- **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	-	128.1	-	-
	2x	74.4	64.0	116.1
	4x	32.8	32.0	102.4
2	-	142.9	-	-
	2x	82.6	71.4	115.7
	4x	39.1	35.7	109.4

- **Effect of sample matrix**

Heparin, citrate and EDTA 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	58.3	47.7	44.5	19.7
2	31.6	25.5	25.6	10.1
3	66.9	54.3	58.0	28.4
4	86.6	64.9	67.7	37.1
5	76.1	62.8	66.7	35.6
6	81.3	61.5	63.3	25.5
7	47.2	35.0	38.7	25.9
8	41.8	31.8	35.4	14.7
9	105.4	82.3	101.2	49.2
10	44.5	30.3	34.9	14.4
Mean (ng/ml)	**64.0**	**49.6**	**53.6**	**26.0**
Mean Plasma/Serum (%)		**78**	**84**	**41**
Coefficient of determination R^2		**0.980**	**0.930**	**0.760**

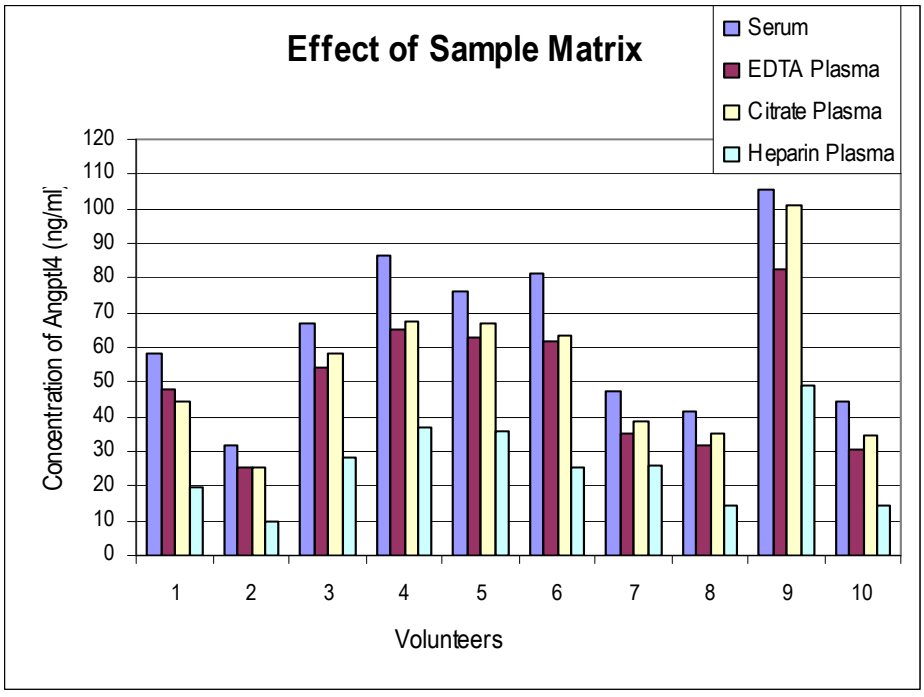


Figure 3: Angptl4 levels measured using Human Angptl4 ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

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

Samples should be stored at -20°C. However, no significant decline in concentration of human Angptl4 was observed in serum 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	97.2	79.9	78.6	29.4
	2-8°C, 1 day	99.7	66.0	82.5	34.2
	2-8°C, 7 days	79.6	66.1	77.4	32.8
2	-20°C	59.6	38.2	34.3	20.9
	2-8°C, 1 day	58.6	24.8	40.0	21.7
	2-8°C, 7 days	47.0	27.4	46.6	31.1
3	-20°C	142.2	130.9	124.4	66.0
	2-8°C, 1 day	142.7	119.6	129.0	63.0
	2-8°C, 7 days	141.7	124.5	140.8	67.7

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human Angptl4 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	58.3	47.7	44.5	19.7
	3x	59.9	49.9	50.9	18.5
	5x	61.0	49.8	47.6	20.8
2	1x	66.9	54.3	58.0	28.4
	3x	65.3	55.5	60.7	27.7
	5x	70.6	59.6	62.7	29.5
3	1x	86.6	64.9	67.7	37.1
	3x	85.4	67.4	73.5	36.9
	5x	94.1	71.3	82.2	39.1

14. DEFINITION OF THE STANDARD

The recombinant human Angptl4 is used as the Standard. Recombinant human Angptl4, produced on HEK293, is 44.2 kDa protein consisting of 392 amino-acid residues of human Angptl4 and 11 additional amino-acids.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 155 unselected donors (89 men + 66 women) 20 - 69 years old were assayed with the BioVendor Human Angptl4 ELISA in our laboratory.

- **Age and Sex dependent distribution of Angiopoietin-like Protein 4**

Sex	Age (years)	n	Mean	SD	Min	Max
			Angptl4 (ng/ml)			
Men	20-39	42	59.5	21.4	32.4	139.8
	40-69	47	64.2	19.4	28.4	103.7
Women	20-39	38	81.2	38.2	37.0	177.6
	40-69	28	56.6	24.9	22.2	124.9

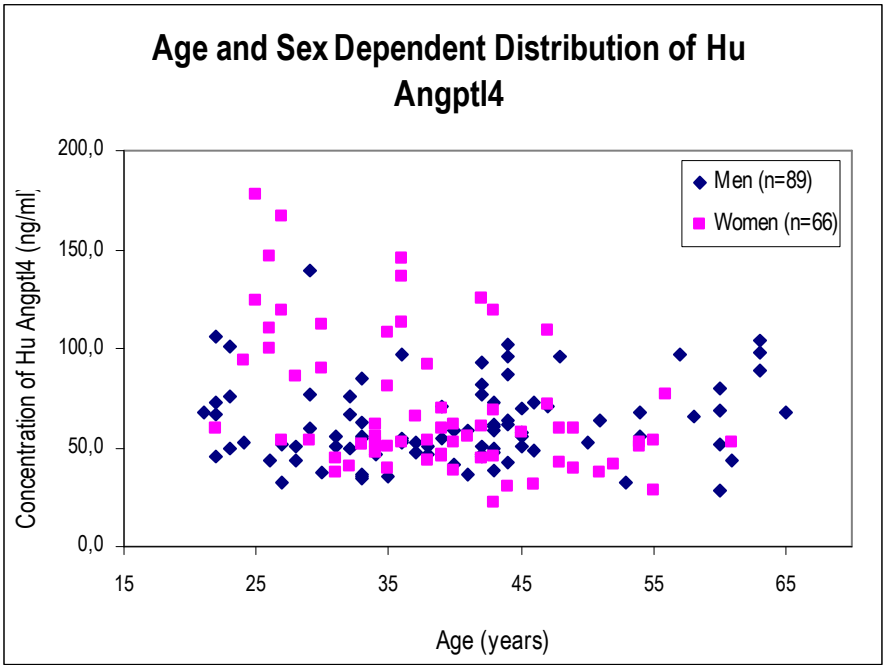


Figure 4: Human Angptl4 concentration plotted against donor age and sex.

- **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 Angptl4 levels with the assay.

16. METHOD COMPARISON

The BioVendor Human Angiopoietin-like Protein 4 ELISA has not been compared to any commercial immunoassay.

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 or samples

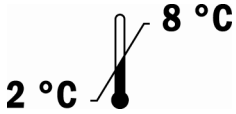
18. REFERENCES

References to Angiopoietin-like Protein 4 :

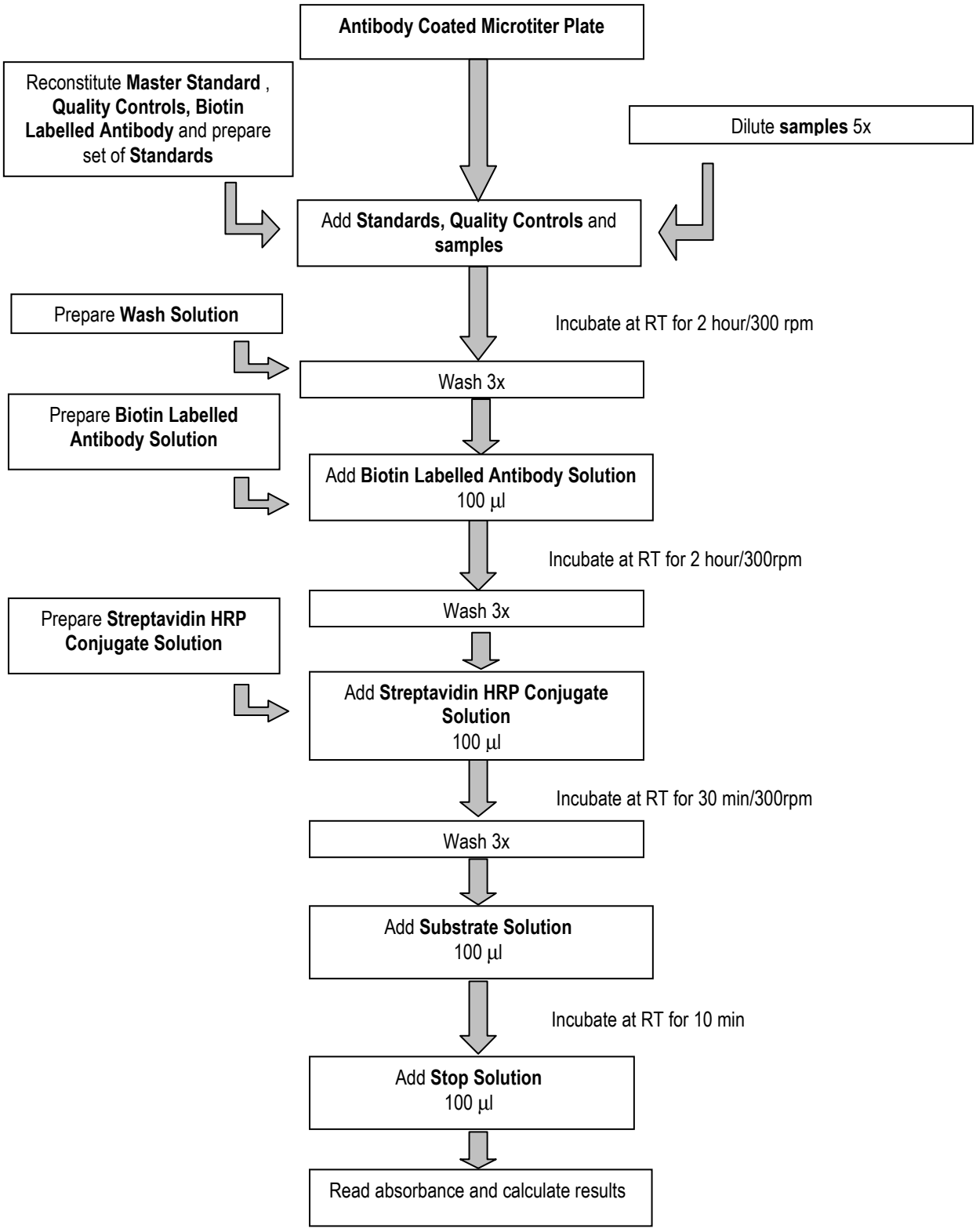
- Hermann LM, Pinkerton M, Jennings K, Yang L, Grom A, Sowders D, Kersten S, Witte DP, Hirsch R, Thornton S. Angiopoietin-like-4 is a potential angiogenic mediator in arthritis. Clin Immunol. 2005 Apr;115(1):93-101.
- Le Jan S, Amy C, Cazes A, Monnot C, Lamandé N, Favier J, Philippe J, Sibony M, Gasc JM, Corvol P, Germain S.: Angiopoietin-like 4 is a proangiogenic factor produced during ischemia and in conventional renal cell carcinoma. Am J Pathol. 2003 May;162(5):1521-8.
- Galaup A, Cazes A, Le Jan S, Philippe J, Connault E, Le Coz E, Mekid H, Mir LM, Opolon P, Corvol P, Monnot C, Germain S.: Angiopoietin-like 4 prevents metastasis through inhibition of vascular permeability and tumor cell motility and invasiveness. Proc Natl Acad Sci U S A. 2006 Dec 5;103(49):18721-6. Epub 2006 Nov 27.
- Sukonina V, Lookene A, Olivecrona T, Olivecrona G.: Angiopoietin-like protein 4 converts lipoprotein lipase to inactive monomers and modulates lipase activity in adipose tissue. PNAS November 14, 2006 vol. 103 no. 46 17450-17455
- Xu A., Lam M. C., Chan K. W., Wang Y., Zhang J., Hoo R. L., Xu J. Y., Chen B., Chow W. S., Tso A. W., Lam K. S. (2005): Angiopoietin-like protein 4 decreases blood glucose and improves glucose tolerance but induces hyperlipidemia and hepatic steatosis in mice. Proc. Natl. Acad. Sci. U.S.A. 102, 6086–6091
- Stejskal D, Karpísek M, Reutová H, Humenanská V, Petzel M, Kusnierová P, Vareka I, Vareková R, Stejskal P. Angiopoietin-like protein 4: development, analytical characterization, and clinical testing of a new ELISA. Gen Physiol Biophys. 2008 Mar;27(1):59-63.
- Robciuc MR, Tahvanainen E, Jauhiainen M, Ehnholm C. Quantitation of serum angiopoietin-like proteins 3 and 4 in a Finnish population sample. J Lipid Res. 2010 Apr;51(4):824-31. Epub 2009 Oct 13.
- Kersten S, Lichtenstein L, Steenbergen E, Mudde K, Hendriks HF, Hesselink MK, Schrauwen P, Müller M. Caloric restriction and exercise increase plasma ANGPTL4 levels in humans via elevated free fatty acids. Arterioscler Thromb Vasc Biol. 2009 Jun;29(6):969-74. Epub 2009 Apr 2.
- Cazes A, Galaup A, Chomel C, Bignon M, Bréchot N, Le Jan S, Weber H, Corvol P, Muller L, Germain S, Monnot C. Extracellular matrix-bound angiopoietin-like 4 inhibits endothelial cell adhesion, migration, and sprouting and alters actin cytoskeleton. Circ Res. 2006 Nov 24;99(11):1207-15. Epub 2006 Oct 26.
- Folsom AR, Peacock JM, Demerath E, Boerwinkle E. Variation in ANGPTL4 and risk of coronary heart disease: the Atherosclerosis Risk in Communities Study. Metabolism. 2008 Nov;57(11):1591-6.

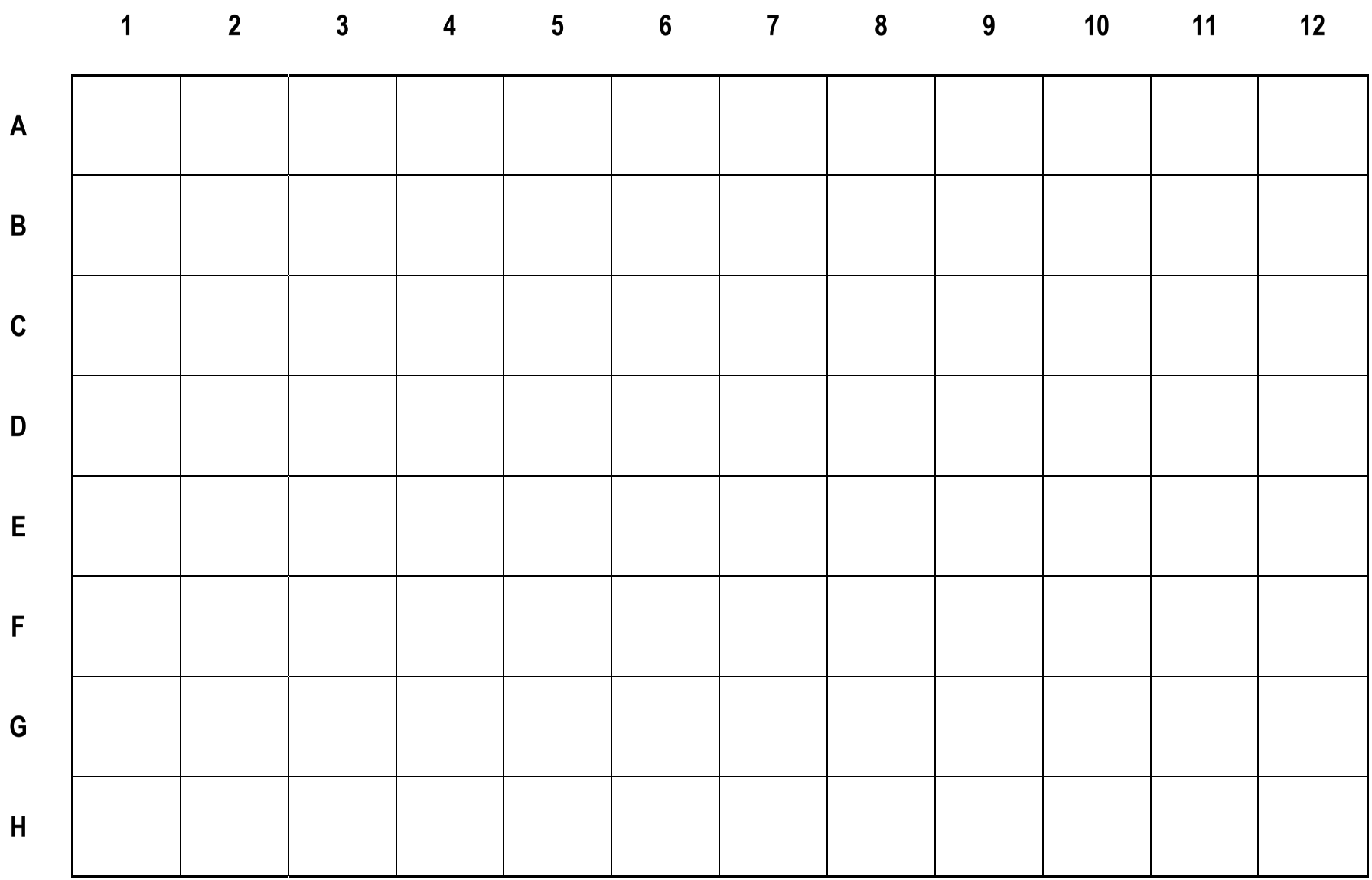
For more references on this product see our WebPages at www.biovendor.com

19. EXPLANATION OF SYMBOLS

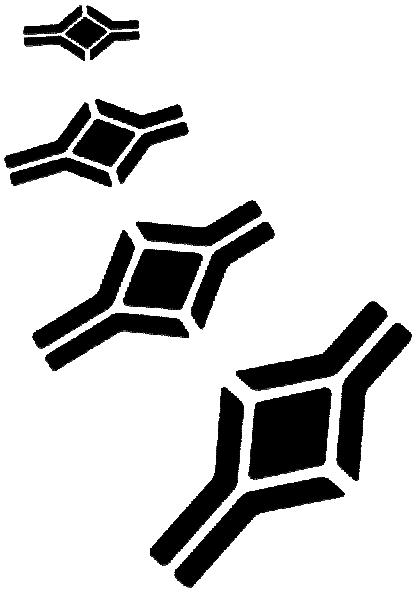
REF	Catalogue number
Cont.	Content
LOT	Lot number
	See instructions for use
	Biological hazard
	Expiry date
	Storage conditions
	Identification of packaging materials

Assay Procedure Summary




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

BioVendor
Research
and Diagnostic Products



HEADQUARTERS: **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**
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, N. 2 Huaqiang road	Pearl River New Town Guang Zhou, CHINA	Phone: +86-20-8706-3029 Fax: +86-20-8706-3016	E-mail: infoCN@biovendor.com