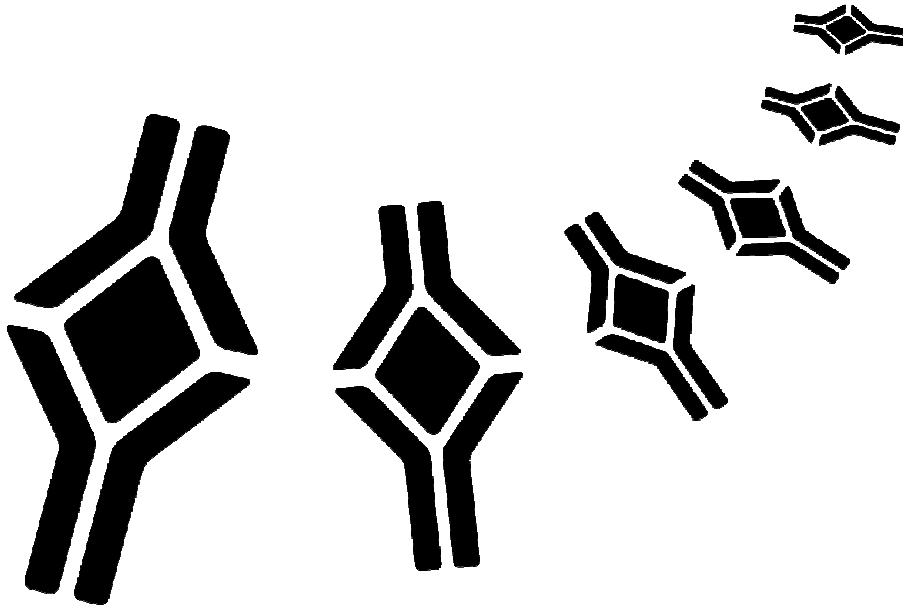# BioVendor
Research and Diagnostic Products

HUMAN ANGIOPOIETIN-LIKE PROTEIN 3 ELISA

Product Data Sheet

Cat. No.: RD191092200R

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 RD191092200R Human Angiopoietin-like Protein 3 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human angiopoietin-like protein 3 (Angptl3).

›› Features

- **It is intended for research use only**
- The total assay time is less than 3.5 hours
- The kit measures Angptl3 in serum and plasma (EDTA, citrate, 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

Angiopoietin-like Protein 3 (Angptl3) is one from six members of Angiopoietin-like (Angptl) family of proteins, which has been identified as orphan ligands with structural similarity to angiopoietins. Angptl3 has 490-amino acid residues and its expression is restricted to the liver. Angptl3 and Angptl4 have been shown to regulate fat, lipid or glucose metabolic homeostasis. Angptl3 is a hepatocyte-derived circulating factor that affect lipid metabolism and is involved in regulating lipid storage and breakdown.

Overexpression of Angptl3 or intravenous injection of the purified protein in mice elicited an increase in circulating plasma lipid levels. Angptl3 decreases very low density lipoprotein (VLDL) triglyceride clearance by inhibiting lipoprotein lipase (LPL) activity, directly targeting adipose cells to activate lipolysis. This results in an increased release of free fatty acid (FFA) and glycerol from adipocytes. These observations indicate that Angptl3 might regulate lipid metabolism by inhibiting LPL and by stimulating lipolysis in adipocytes.

Recent findings suggest that elevated levels of Angptl3 in diabetic states might be involved in inducing hypertriglyceridemia and hyperfattyacidemia in diabetes and obesity. Hypertriglyceridemia causes triglyceride accumulation in peripheral tissues such as skeletal muscles to enhance insulin- and leptin- resistance, and in vessel walls to induce atherosclerosis. Hyperfattyacidemia also affects the regulation of insulin secretion.

Taken together, these findings suggest that abnormalities in the regulation of Angptl3 might be involved in the pathogenesis of metabolic syndrome.

Angptl3 also suggest a possible role in the regulation of proliferation of new vessel from preexisting capillaries; a process termed angiogenesis also plays a key role in the progression of solid tumor growth, diabetic retinopathies, psoriasis, inflammation, and rheumathoid arthritis.

Areas of investigation:
Lipid metabolism
Angiogenesis
Atherosclerosis

4. TEST PRINCIPLE

In the BioVendor Human Angiopoietin-like Protein 3 ELISA, the standards, quality controls and samples are incubated in microtitrate wells pre-coated with polyclonal anti-human Angptl3 antibody. After 60 min incubation and a washing, biotin labelled polyclonal anti-human Angptl3 antibody is added and incubated with captured Angptl3 for 60 min. After another washing, the streptavidin-HRP conjugate is added. After 30 min 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 Angptl3. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

5. PRECAUTIONS

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

6.   TECHNICAL HINTS

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

7.   REAGENT SUPPLIED

Kit Components	State	Quantity
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody Conc. (100x)	concentrated	0.15 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	1 vial
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
Dilution Buffer	ready to use	20 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
Instruction Manual + 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 $\pm$ 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 desicant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8°C and protected from the moisture.

Biotin-Ab Diluent
Streptavidin-HRP Conjugate
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 Angptl3 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 Angptl3 in the stock solution is **400 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	-	400 ng/ml
250 µl of stock	250 µl	200 ng/ml
250 µl of 200 ng/ml	250 µl	100 ng/ml
250 µl of 100 ng/ml	250 µl	50 ng/ml
250 µl of 50 ng/ml	250 µl	25 ng/ml
250 µl of 25 ng/ml	250 µl	12.5 ng/ml

Prepared Standards are ready to use, do not dilute them.
Stability and storage:
Reconstituted Master Standard must be used immediately or stored frozen at –20°C for 3 months. Avoid repeated freeze/thaw cycles.
Do not store the diluted 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:
The Quality Control has to be reconstituted exactly before assay.
Do not store the reconstituted Quality Controls.

Note:
Concentration of analyte in Quality Control need not be anyhow associated with normal and/or pathological concentrations in serum or another body fluid. Quality Control serves 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. (100x)
Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (100x) with 99 parts Biotin-Ab Diluent. Example: 10 μl of Biotin Labelled Antibody Concentrate (100x) + 990 μl of Biotin-Ab Diluent for 1 strip (8 wells).
<u>Stability and storage:</u>
Opened Biotin Labelled Antibody Concentrate (100x) 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.
<u>Stability and storage:</u>
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 Angptl3 in serum and plasma (EDTA, citrate, heparin)

Samples should be assayed immediately after collection or should be stored at -20°C. Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Dilute serum or plasma samples 5x with Dilution Buffer just prior to the assay, e.g. 30 μl of sample + 120 μl of Dilution Buffer for singlets, or preferably 50 μl of sample + 200 μl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.
<u>Stability and storage:</u>
Samples have to be diluted exactly before assay.
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 Angptl3.

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 diluted Standards, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells. See *Figure 1* for example of work sheet.
2. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add **100 µl** of 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 minutes**, 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.**

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 Angptl3 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 400**	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 200**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 100**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 50**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 25**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 12.5**	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 Angptl3 (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 and calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay, e.g. 45.9 ng/ml (from standard curve) x 5 (dilution factor) = 229.5 ng/ml.
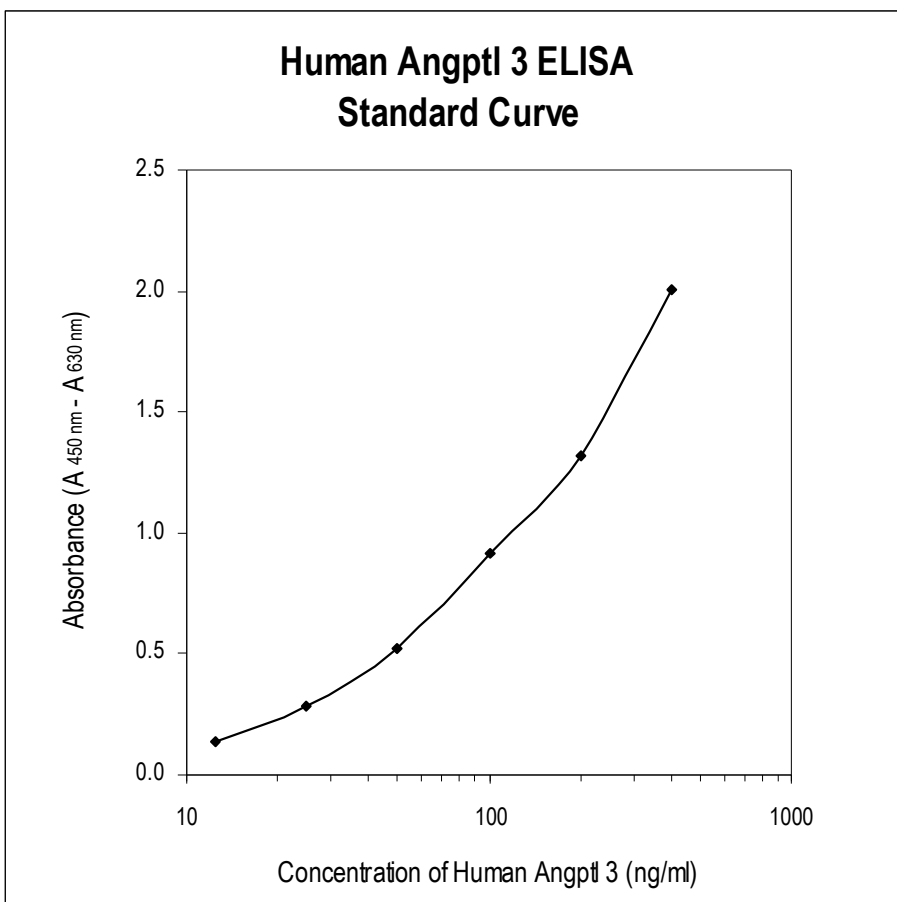

Figure 2: Typical Standard Curve for Human Angptl3 ELISA.

13.   PERFORMANCE CHARACTERISTICS

➤➤   Typical analytical data of BioVendor Human Angiopoietin-like Protein 3 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: $A_{blank} + 3 \times SD_{blank}$) is calculated from the real Angptl3 values in wells and is 1.08 ng/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding Angptl3 level of 400 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the Angptl3 concentration.

- **Specificity**

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

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	yes
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)	Standard Deviation (ng/ml)	CV (%)
1	775.7	14.1	1.8
2	1238.2	69.0	5.6

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

Sample	Mean (ng/ml)	Standard Deviation (ng/ml)	CV (%)
1	355.8	37.5	10.5
2	545.8	39.7	7.3

- **Spiking Recovery**

Serum samples were spiked with different amounts of human Angptl3 and assayed.

Sample	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
1	210.4	-	-
	1023.0	1210.4	84.5
	661.1	710.4	93.1
	445.8	460.4	96.8
2	395.6	-	-
	1417.9	1395.6	101.6
	887.4	896.6	99.1
	643.8	645.6	99.7

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
1	-	706.5	-	-
	2x	363.3	353.2	102.8
	4x	192.9	176.6	109.2
	8x	94.9	88.3	107.5
2	-	1223.4	-	-
	2x	611.8	611.7	100.0
	4x	298.7	305.8	97.7
	8x	146.6	152.9	95.9

ENG.001.A

- **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 heparin plasma Angptl3 values. Results are shown below:

| Individuals No.	Concentration of Angptl3 (ng/ml)			
	Serum	Plasma		
		EDTA	Citrate	Heparin
1	572.9	580.7	579.1	656.0
2	600.2	574.6	463.2	372.6
3	468.1	550.3	488.9	547.6
4	581.6	514.3	534.1	476.0
5	464.6	457.7	405.4	356.0
6	319.1	314.4	287.7	237.3
7	529.6	528.8	511.4	434.8
8	214.4	200.6	147.1	167.9
9	363.8	401.2	258.4	274.9
10	343.9	313.9	358.5	291.6
Mean (ng/ml)	**445.8**	**443.7**	**403.4**	**381.5**
Mean Plasma/Serum	**-**	**99.5%**	**90.5%**	**85.6%**
Coefficient of determination R^2		**0.90**	**0.90**	**0.60**
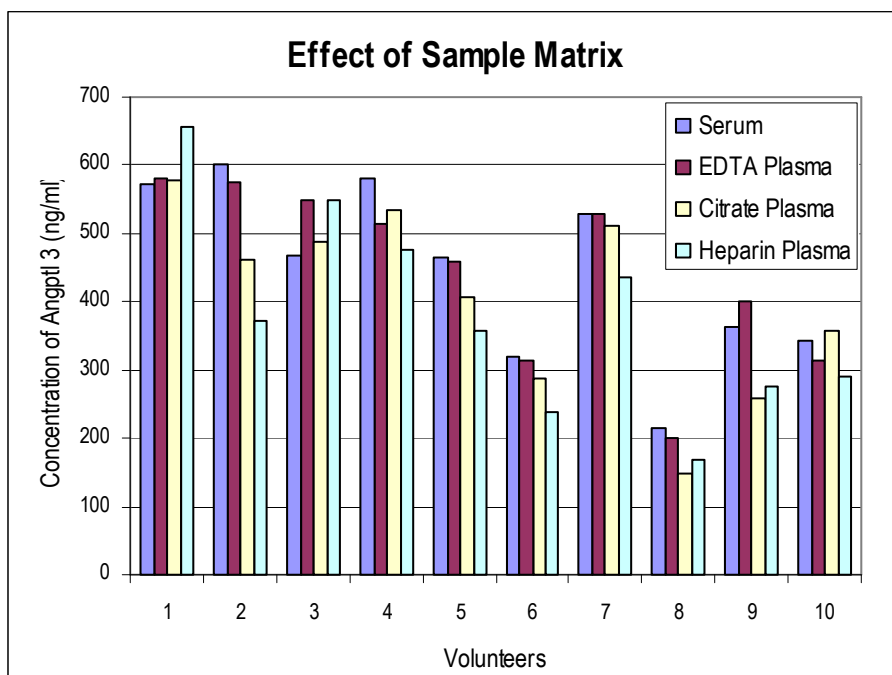
Figure. 3: Angptl3 levels measured using Human Angiopoietin-like Protein 3 ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

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

Samples should be stored at -20°C. However, no decline in concentration of Angptl3 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 thimerosal, resulting in the final concentration of 0.03% and 0.05%, respectively.

Sample	Incubation Temp, Period	Serum (ng/ml)	Plasma (ng/ml)		
			EDTA	Citrate	Heparin
1	-20°C	253.8	239.9	214.5	202.5
	2-8°C, 1 day	340.4	249.8	255.6	266.3
	2-8°C, 7 days	304.2	268.1	227.9	230.3
2	-20°C	527.1	483.5	422.3	422.9
	2-8°C, 1 day	503.1	496.2	418.9	341.7
	2-8°C, 7 days	558.5	508.0	459.9	412.8
3	-20°C	275.1	253.8	235.8	244.6
	2-8°C, 1 day	317.8	281.3	245.7	326.1
	2-8°C, 7 days	283.5	261.6	215.8	241.4

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human Angptl3 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	589.1	584.1	532.5	543.4
	3x	575.2	617.1	522.9	560.9
	5x	591.8	609.3	519.9	570.2
2	1x	501.5	493.2	415.2	432.1
	3x	497.2	465.6	408.3	431.8
	5x	489.9	522.9	399.3	502.3
3	1x	280.9	282.9	242.8	229.2
	3x	278.9	289.2	257.5	271.4
	5x	342.3	280.9	225.7	278.5

- **Diurnal Variation**

Diurnal variation of Angptl3 levels in serum was determined in 4 patients in course of 24 hours.

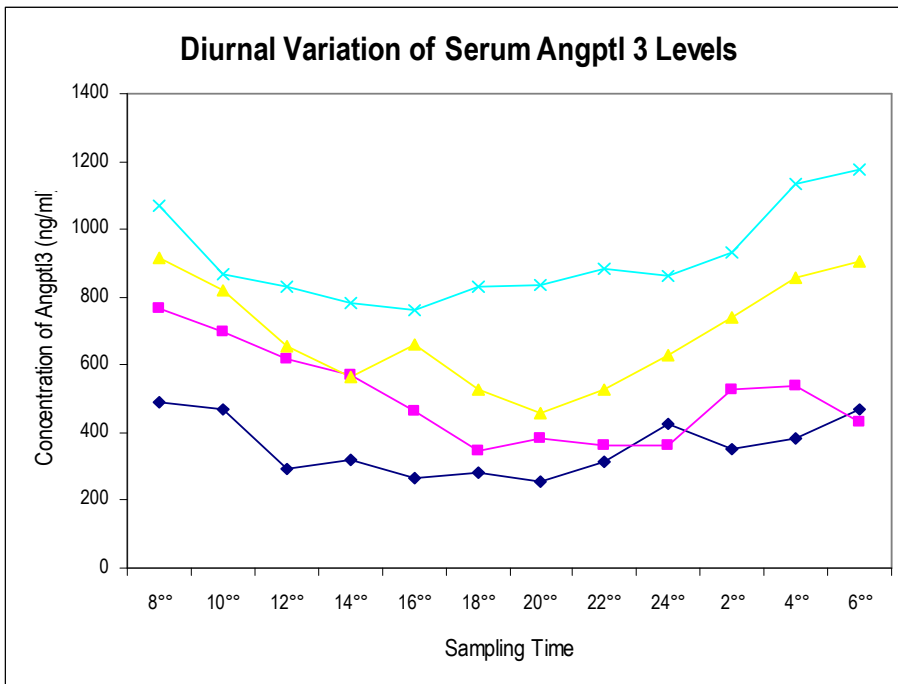**Diurnal Variation of Serum Angptl 3 Levels**

Figure 4: Diurnal variation of serum Angptl3 levels.

14.   DEFINITION OF THE STANDARD

The recombinant protein is used as a Standard in this assay. The recombinant Angptl3 is 207 amino acid residues protein produced in *E. coli*. The molecular weight is 26 kDa.

15.   PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum from 141 unselected donors (69 women + 72 men), 10-99 years old were assayed with Biovendor Human Angiopoietin-like Protein 3 ELISA kit in our laboratory.

The presented data should be regarded as guideline only.

- **Age and sex - dependent distribution of Angptl3**

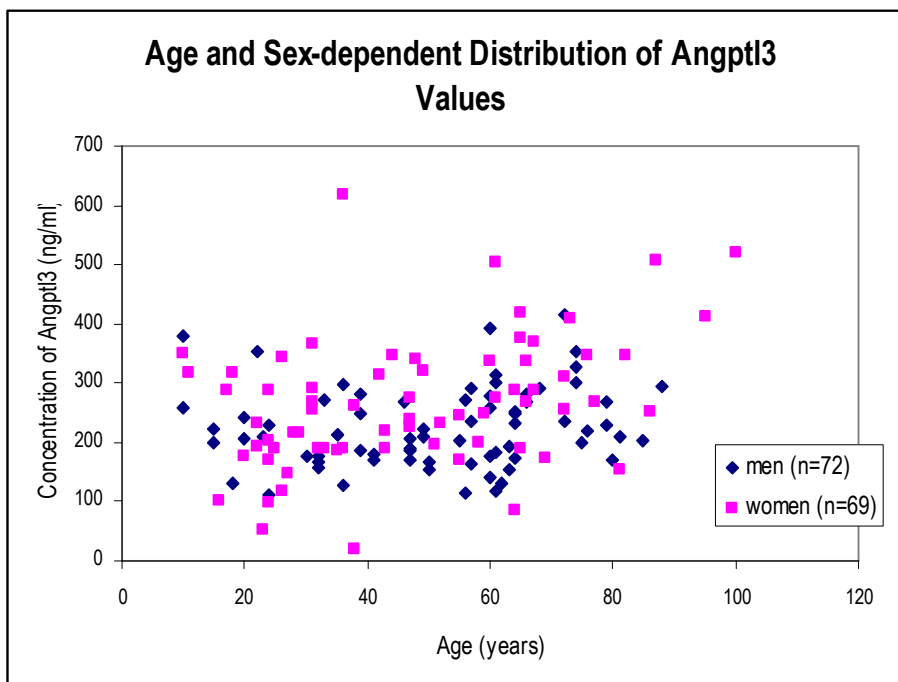| Sex	Age years	n	Mean	SD	Min.	Max.	Median
			ng/ml				
Men	10-18	5	237.8	82.05	129.7	378.3	222.4
	20-49	27	210.1	51.83	112.6	351.9	206.3
	50-88	40	235.6	71.58	116.1	416.5	232.8
Women	9-19	5	274.7	88.79	101.3	349.3	316.3
	20-49	33	240.3	98.27	53.3	617.4	217.8
	50-99	31	299.5	106.29	86.6	518.5	280.9

Figure 5: Angptl3 concentration plotted against donor age.

- **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 references ranges for Angiopoietin-like Protein 3 levels with the assay.

16. METHOD COMPARISON

The BioVendor Human Angiopoietin-like Protein 3 ELISA was compared to the other commercial immunoassay by measuring 14 serum samples. The following correlation graph was obtained.
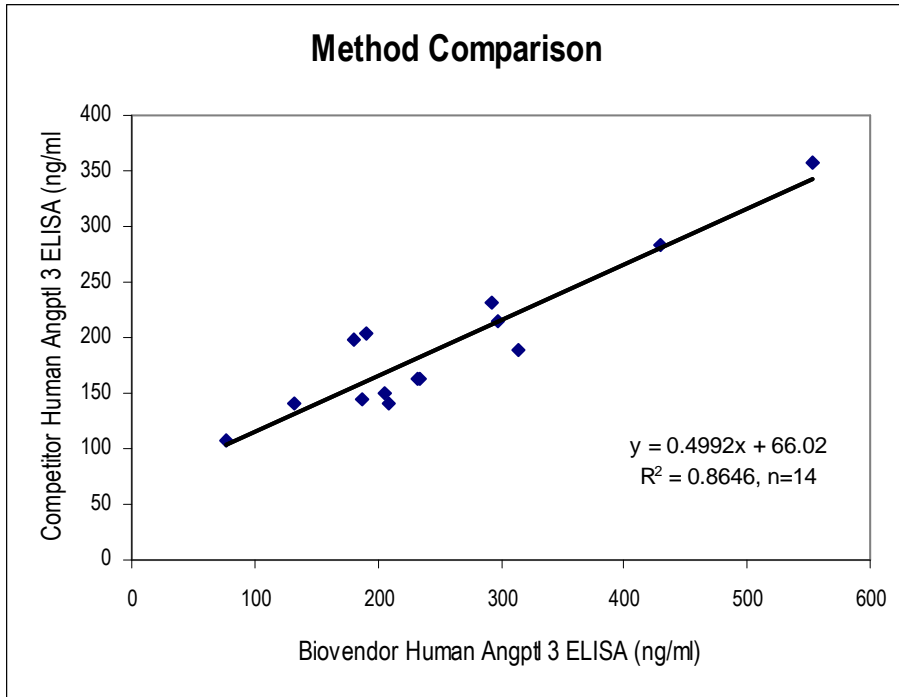

Method Comparison

$y = 0.4992x + 66.02$
$R^2 = 0.8646, n=14$

Figure 6: 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

» References to Angiopoietin-like Protein 3:

- Hatsuda S., Shoji T., Shinohara K., Kimoto E., Mori K., Fukumoto S., Koyama H., Emoto M., Nishizawa Y.: Association between plasma angiopoietin-like protein 3 and arterial wall thickness in healthy subjects. J Vasc Res. 2007;44(1):61-6.
- Fujimoto K., Koishi R., Shimizugawa T., Ando Y.: Angptl3-null mice show low plasma lipid concentrations by enhanced lipoprotein lipase activity. Exp Anim. 2006; 55(1):27-34
- Shimamura M, Matsuda M, Yasumo H, Okazaki M, Fujimoto K, Kono K, Shimizugawa T, Ando Y, Koishi R, Kohama T, Sakai N, Kotani K, Komuro R, Ishida T, Hirata K, Yamashita S, Furukawa H, Shimomura I. Angiopoietin-like protein3 regulates plasma HDL cholesterol through suppression of endothelial lipase. Arterioscler Thromb Vasc Biol. 2007;27(2):366-72
- Dormer A, Beck G. Evolutionary analysis of human vascular endothelial growth factor, angiopoietin, and tyrosine endothelial kinase involved in angiogenesis and immunity. In Silico Biol. 2005;5(3):323-39
- Ge H., Cha J.Y., Gopal H., Harp Ch., Yu X., Repa J.J. and Li C.: Differential regulation and properties of angiopoietin-like proteins 3 and 4. J Lipid Res. 2005; 46(7):1484-90
- Kersten S. Regulation of lipid metabolism via angiopoietin-like proteins. Biochem Soc Trans. 2005;33(Pt 5):1059-62
- Oike Y., Akao M., Kubota Y. and Suda T.: Angiopoietin-like proteins: potential new targets for metabolic syndrome therapy. Trends Mol Med. 2005; 11(10):473-9.
- Ando Y., Shimizugawa T., Takeshita S., Ono M., Shimamura M., Koishi R., Furukawa H.: A decreased expression of angiopoietin-like 3 is protective against atherosclerosis in apoE-deficient mice. J Lipid Res. 2003; 44(6):1216-23
- Inaba T., Matsuda M., Shimamura M., Takei N., Terasaka N., Ando Y., Yasumo H., Koishi R., Makishima M., Shimomura I.: Angiopoietin-like protein 3 mediates hypertriglyceridemia induced by the liver X receptor. J Biol Chem. 2003; 278(24):21344-51
- Kaplan R., Zhang T., Hernandez M., Gan F.X., Wright S.D., Waters M.G. and Cai T.-Q.: Regulation of the angiopoietin-like protein 3 gene by LXR. J Lipid Res. 2003; 44(1):136-43
- Ono M., Shimizugawa T., Shimamura M., Yoshida K., Noji-Sakikawa C., Ando Y., Koishi R., Furukawa H.: Protein region important for regulation of lipid metabolism in angiopoietin-like 3 (ANGPTL3): ANGPTL3 is cleaved and activated in vivo. J Biol Chem. 2003; 278(43):41804-9
- Camenisch G, Pisabarro MT, Sherman D, Kowalski J, Nagel M, Hass P, Xie MH, Gurney A, Bodary S, Liang XH, Clark K, Beresini M, Ferrara N, Gerber HP. ANGPTL3 stimulates endothelial cell adhesion and migration via integrin alpha vbeta 3 and induces blood vessel formation in vivo. J Biol Chem. 2002 10;277(19):17281-90
- Koishi R., Ando Y., Ono M., Shimamura M., Yasumo H., Fujiwara T., Horikoshi H., Furukawa H.: Angptl3 regulates lipid metabolism in mice. Nat Genet. 2002; 30(2):151-7

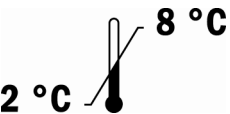- Shimizugawa T., Ono M., Shimamura M., Yoshida K., Ando Y., Koishi R., Ueda K., Inaba T., Minekura H., Kohama T., Furukawa H.: ANGPTL3 decreases very low density lipoprotein triglyceride clearance by inhibition of lipoprotein lipase. J Biol Chem. 2002; 277(37):33742-8
- Yoon J.C., Chickering T.W., Rosen E.D., Dussault B., Qin Y., Soukas A., Friedman J.M., Holmes W.E.: Peroxisome proliferator-activated receptor gamma target gene encoding a novel angiopoietin-related protein associated with adipose differentiation. Mol Cell Biol. 2000; 20(14):5343-9
- Conklin D., Gilbertson D., Taft D.W., Maurer M.F., Whitmore T.E., Smith D.L., Walker K.M., Chen L.H., Wattler S., Nehls M., Lewis K.B.Y: Identification of a mammalian angiopoietin-related protein expressed specifically in liver. Genomics. 1999; 62(3):477-82

❱❱ **References to this product:**

- Stejskal D, Karpísek M, Humenanská V, Solichová P, Stejskal P.: Angiopoietin-like protein 3: development, analytical characterization, and clinical testing of a new ELISA.. Gen Physiol Biophys. 2007 Sep;26(3):230-3

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

19. EXPLANATION OF SYMBOLS

Symbol	Meaning
REF	Catalogue number
Cont.	Content
LOT	Lot number
⚠	See instructions for use
☣	Biological hazard
⧗	Expiry date
2 °C – 8 °C	Storage conditions
♲ 5 PP	Identification of packaging materials

Assay Procedure Summary

Antibody Coated Microtiter Strips

Reconstitute **QCs** and **Master Standard** and prepare set of **Standards**

Dilute **samples** 5x

Add **Standards**, **QCs** and **samples**
100 µl

Incubate at RT for 1 hour/ 300 rpm

Prepare **Wash Solution**

Wash 3x

Prepare **Biotin Labelled Ab solution**

Add **Biotin Labelled Ab solution**
100 µl

Incubate at RT for 1 hour/ 300 rpm

Wash 3x

Add **Streptavidin-HRP Conjugate**
100 µl

Incubate at RT for 30 min/ 300 rpm

Wash 3x

Add **Substrate Solution**
100 µl

Incubate at RT for 10 min

Add **Stop Solution**
100 µl

Read absorbance and calculate results

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

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