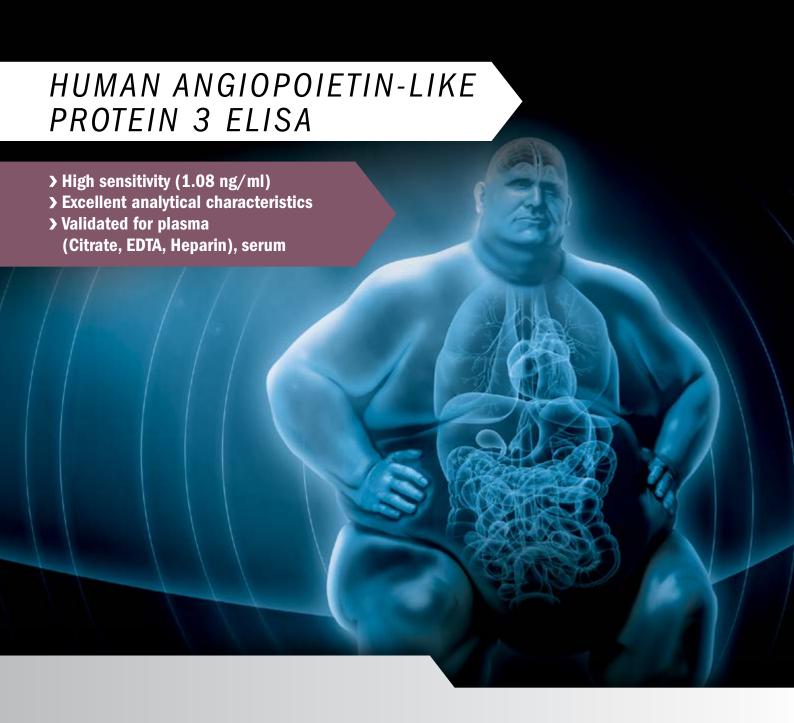
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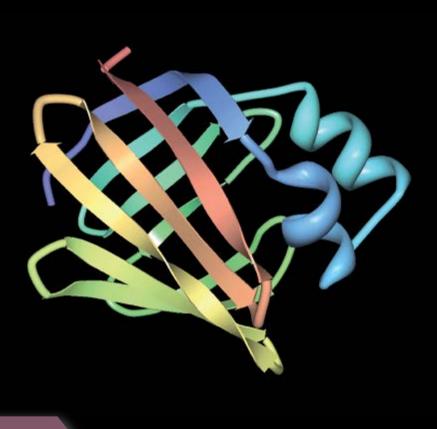
QUANTITATIVE DETERMINATION OF HUMAN ANGIOPOIETIN-LIKE PROTEIN 3



ENERGY METABOLISM AND BODY WEIGHT REGULATION ONCOLOGY



HUMAN ANGIOPOIETIN-LIKE PROTEIN 3 ELISA



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.

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BioVendor Human Angiopoietin-like Protein 3 ELISA (RD191092200R)

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).

> 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

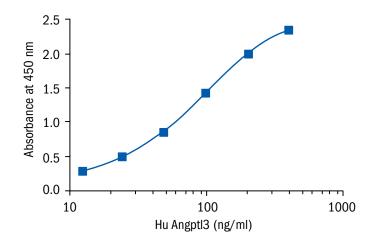
Clinical application

- Energy metabolism and body weight regulation
- Oncology

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.

HUMAN ANGIOPOIETIN-LIKE PROTEIN 3 ELISA CAT. NO.: RD191092200R			
Assay format	Sandwich ELISA, Biotin-labelled antibody, 96 wells/kit		
Samples	Serum, plasma (EDTA, citrate, heparin)		
Standards	12.5 to 400 ng/ml		
Limit of detection	1.08 ng/ml		



HUMAN ANGIOPOIETIN-LIKE PROTEIN 3 ELISA

Precision

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

Sample	Mean	SD	CV
	(ng/ml)	(ng/ml)	(%)
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)	SD (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	Sample Observed (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

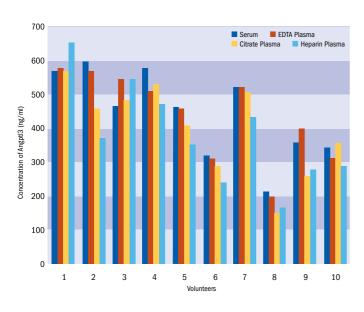
Linearity

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

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:



Volunteer	Serum	Plasma (ng/ml)		
No.	(ng/ml)	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

QUANTITATIVE DETERMINATION OF HUMAN ANGIOPOIETIN-LIKE PROTEIN 3

Summary of protocol

- · Reconstitute QCs and Master Standard and prepare set of Standards
- · Dilute samples 5.
- · Add 100 µl Standards, QCs and samples
- · Incubate at RT for 1 hour/300 rpm
- · Wash plate 3 times
- · Add 100 µl Biotin Labelled Ab solution
- · Incubate at RT for 1 hour/300 rpm
- · Wash plate 3 times
- · Add 100 µl Streptavidin-HRP Conjugate
- · Incubate at RT for 30 min/300 rpm
- · Wash plate 3 times
- · Add 100 µl Substrate Solution
- · Incubate at RT for 10 min
- · Add 100 µl Stop Solution
- · Read absorbance and calculate results

Related products

- · RAG011R Angiopoietin-Like Protein 3 Mouse/Rat ELISA
- · RD172092100 Angiopoietin-Like Protein 3 Human E. coli
- · RD172092050-HEK Angiopoietin-Like Protein 3 Human HEK293
- · RD181092100 Angiopoietin-Like Protein 3 Human, Rabbit Polyclonal Antibody
- · RD184092100 Angiopoietin-Like Protein 3 Human, Sheep Polyclonal Antibody

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