

A SANDWICH ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF HUMAN ANGPTL4

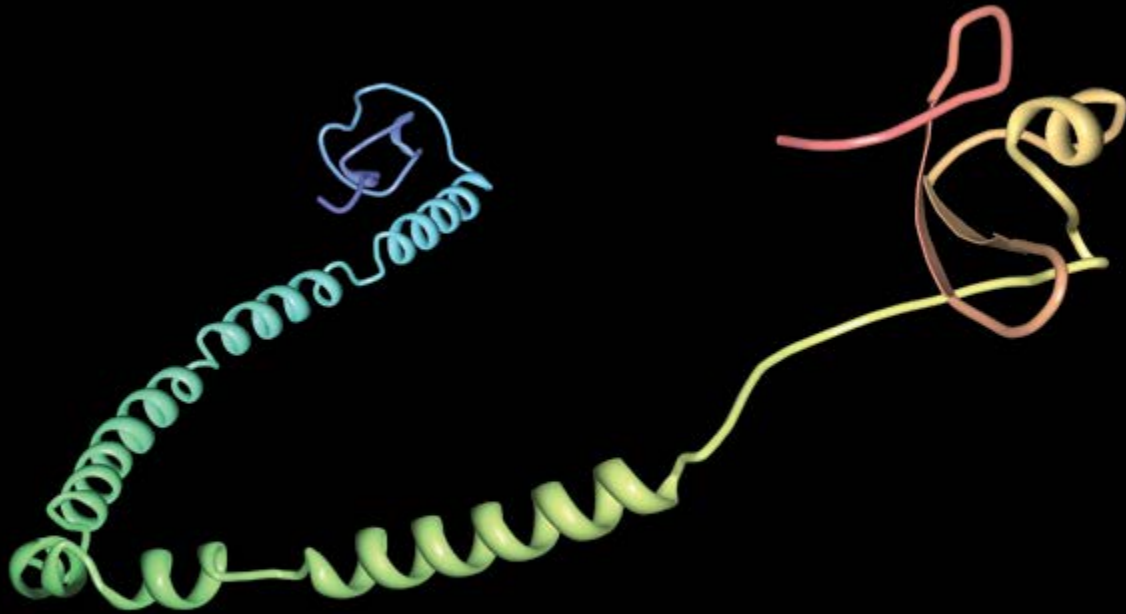
Human Angiopoietin-Like Protein 4 ELISA

BioVendor Research and Diagnostic Products releases its new Human Angiopoietin-Like Protein 4 ELISA. The ANGPTL4 ELISA has been optimized and validated for the quantitative determination of human ANGPTL4 in serum and plasma.



**ENERGY METABOLISM AND
BODY WEIGHT REGULATION**

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Introduction

Angiopoietin-like protein 4 (ANGPTL4) is a secreted 50 kD protein that modulates the disposition of circulating triglycerides (TG) by inhibiting lipoprotein lipase (LPL). ANGPTL4 was identified as a gene that is induced by fasting, and during 3T3-L1 preadipocyte differentiation, and was thus named HFARP (hepatic fibrinogen/angiopoietin-related protein), FIAF (fasting-induced adipose factor), and PGAR (PPAR-angiopoietin related). It is one of the seven members of the angiopoietin-like family. *Angptl4* is expressed ubiquitously, predominantly in adipose tissue, liver, placenta, myocardium, keratinocytes, podocytes, intestine, and pituitary gland. ANGPTL4 is a fusion protein consisting of an N-terminal coiled-coil domain and a C-terminal fibrinogen-like domain. These two domains have been shown to have distinct biological functions. The N-terminal domain is responsible for the inhibitory effects on LPL, converting the active form of LPL into an inactive form, and the C-terminus mediates its antiangiogenic functions. Interestingly, these two domains are separated by a short linker that can be cleaved after secretion. Upon secretion into the circulation, ANGPTL4 is cleaved into an N-terminal domain and a C-terminal fibrinogen-like domain. The N-terminal peptide circulates as an oligomer, and the fibrinogen-like domain circulates as a monomer. The N-terminal domain of ANGPTL4 interacts directly but transiently with LPL, triggering a stable conformational switch

in LPL that irreversibly inactivates the enzyme. Cleavage of ANGPTL4 appears to be tissue-dependent in humans; liver secretes cleaved ANGPTL4, whereas adipose tissue secretes the full-length form. In mice the full-length form of ANGPTL4 is physically associated with HDL, whereas truncated ANGPTL4 is associated with low density lipoprotein. In humans, both full-length and truncated ANGPTL4 are associated with HDL. ANGPTL4 expression is upregulated by fasting, free fatty acids, PARR agonists, acute phase response, glucocorticoids, and downregulated by insulin.

ANGPTL4 has been implicated in a variety of diseases, including cardiovascular disease, cancer metastasis, obesity, diabetes, wound repair, inflammation, arthritis and nephrotic syndrome. Serum or plasma levels were determined in a limited number of studies. ANGPTL4 serum levels display high variability between individuals ranging from 2 to 158 ng/ml. In post-heparin plasma, ANGPTL4 is increased. ANGPTL4 correlates positively with age, body fat mass, waist-hip-ratio and free fatty acids but negatively with plasma high-density lipoprotein cholesterol. No correlation with triglycerides was observed in one study. ANGPTL4 is a positive acute phase protein and its increase could contribute to the hypertriglyceridemia that characteristically occurs during the acute phase response by inhibiting LPL activity.

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BioVendor Human Angiopoietin-Like Protein 4 ELISA (RD191073200R)

Intended use

The RD191073200R Human Angiopoietin-Like Protein 4 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human Angptl4 in serum and plasma.

- The total assay time is less than 5 hours
- The kit measures total Angptl4 in serum and plasma (EDTA, citrate)
- 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

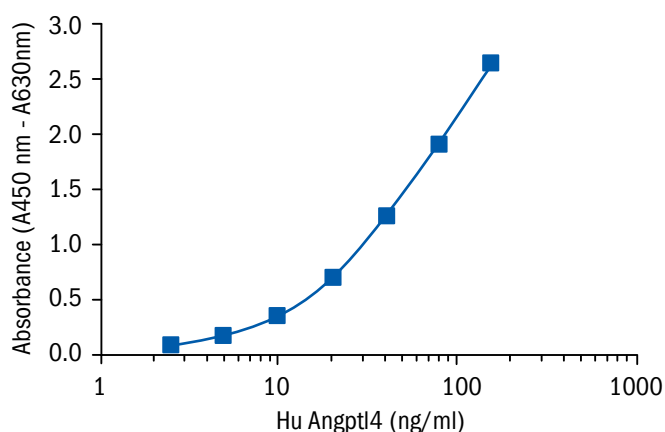
Clinical application

- Energy metabolism and body weight regulation
- Lipid metabolism
- Angiogenesis

Test principle

In the BioVendor Human Angptl4 ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human Angptl4 antibody. After 120 minutes incubation and washing, biotin labelled polyclonal anti-human Angptl4 antibody is added and incubated for 120 minutes with captured Angptl4. 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 concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

HUMAN ANGIOPOIETIN-LIKE PROTEIN 4 ELISA CAT. NO.: RD191073200R	
Assay format	Sandwich ELISA, Biotin-labelled antibody, 96 wells/kit
Samples	Plasma-Citrate, Plasma-EDTA, Serum
Controls	QC-Low, QC-High
Standards	2.5 to 160 ng/ml
Limit of detection	Analytical Limit of Detection is calculated from the real human Angptl4 values in wells and is 1.174 ng/ml.



Precision

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

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	149.4	4.4	2.9
2	245.3	8.9	3.6

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

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	60.0	4.2	6.9
2	229.6	11.0	4.8

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Spiking recovery

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

Sample	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
1	90.68	-	-
	107.78	110.68	97.4
	122.74	130.68	93.9
	150.36	170.68	88.1
2	66.06	-	-
	82.66	86.06	96.1
	98.54	106.06	92.9
	131.13	146.06	89.8

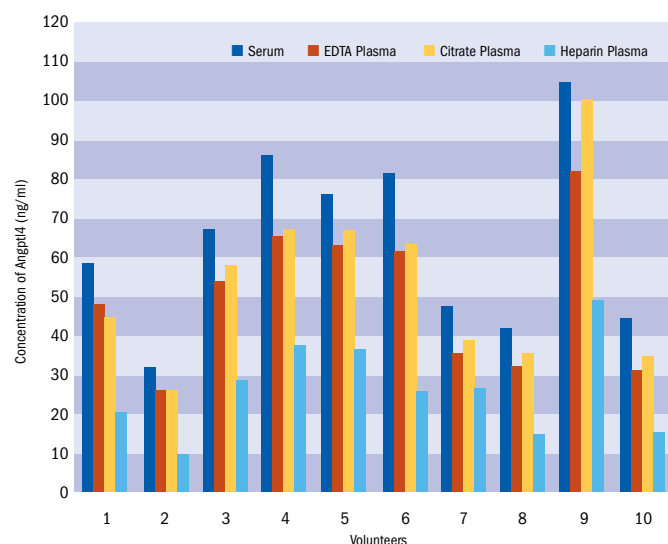
Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
1	-	197.61	-	-
	2×	107.79	98.80	109.1
	4×	53.47	49.40	108.2
	8×	22.32	24.70	90.3
2	-	294.04	-	-
	2×	175.13	147.02	119.1
	4×	88.76	73.51	120.7
	8×	32.54	36.76	88.5

Effect of sample matrix

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



Summary of protocol

- Reconstitute Master Standard and prepare set of Standards
- Reconstitute QCs and dilute samples 5×
- Add 100 μ l Standards, QCs and samples
- Incubate at RT for 2 hours/300 rpm
- Wash plate 3 times
- Add 100 μ l Biotin Labelled antibody
- Incubate at RT for 2 hours/300 rpm
- Wash plate 3 times
- Add 100 μ l Streptavidin-HRP Conjugate
- Incubate at RT for 30 min/300 rpm
- Wash plate 3 times
- Add 100 μ l Substrate Solution
- Incubate at RT for 10 min
- Add 100 μ l Stop Solution
- Read absorbance and calculate results

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Preliminary Population and Clinical Data

Sex	Age (years)	n	Mean (ng/ml)	SD (ng/ml)	Min. (ng/ml)	Max. (ng/ml)	Median (ng/ml)
Men	20-39	59	117.93	44.11	56.89	271.15	114.37
	40-69	47	131.52	42.13	64.04	295.06	126.25
Women	20-39	43	135.12	59.73	43.29	290.48	127.13
	40-69	34	104.06	44.95	41.19	262.70	100.13

Age and Sex Dependent Distribution of Hu ANGPTL4



Related products

- RD172073100 Angiotensin-like Protein 4 Human E. coli
- RD172073100-HEK Angiotensin-like Protein 4 Human HEK293
- RD181073100-01 Angiotensin-like Protein 4 (ANGPTL4) Human, Rabbit Polyclonal Antibody
- RD184073100-01 Angiotensin-like Protein 4 (ANGPTL4) Human, Sheep Polyclonal Antibody
- RD184073100-02 Angiotensin-like Protein 4 (HEK) Human, Sheep Polyclonal Antibody
- RD191092200R Human Angiotensin-Like Protein 3 ELISA
- RAG001R Human Angiotensin-Like Protein 6 ELISA



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