A SANDWICH ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF HUMAN ANGPTL6

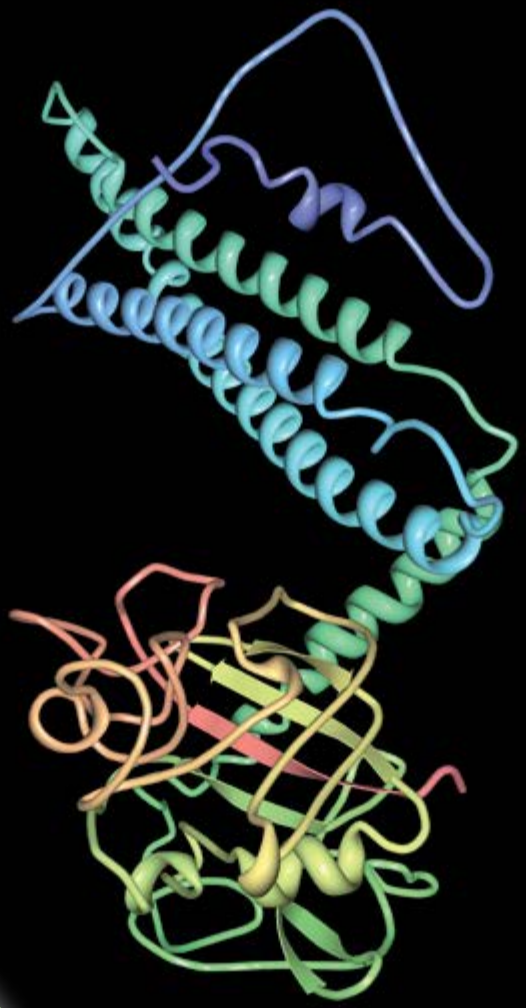
Human Angiopoietin-Like Protein 6 ELISA

BioVendor Research and Diagnostic Products releases its new Human Angiopoietin-Like Protein 6 ELISA. The ANGPTL6 ELISA has been optimized and validated for the quantitative determination of human ANGPTL6 in serum, plasma and cell culture supernatant.

ENERGY METABOLISM AND BODY WEIGHT REGULATION

BioVendor
Research
and Diagnostic Products

A SANDWICH ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF HUMAN ANGPTL6



Introduction

Angiopoietin-like protein 6 (ANGPTL6), which is also called Angiopoietin-like Growth Factor (AGF), is comprised of 470 amino acids, giving rise to a protein of about 50 kDa. ANGPTL6 is a hepatocyte-derived circulating factor that counteracts high-fat diet-induced obesity and related insulin resistance through increased energy expenditure. Mice that survived deletion of the *ANGPTL6* gene showed marked obesity, characterized by elevated fat mass and fat-cell size, hypercholesterolaemia, elevated plasma non-esterified fatty acids, hyperinsulinaemia and glucose intolerance. The increased fat mass could be ascribed to decreased energy expenditure, and is unrelated to food intake.

Mice overexpressing ANGPTL6 in the liver and mice infected with ANGPTL6- expressing adenovirus had high energy expenditure, and, consequently, diminished fat mass. Significant increases in the expression of PPARa, PPARd, PGC-1a and UCP2 in skeletal muscle and of PPARa, PPARg and PGC-1b in brown adipose tissue in AGF-transgenic mice suggest that overexpression of AGF in vivo activates molecules involved in stimulating energy expenditure and thereby leads to decreased adiposity. Morover, the density of capillaries was remarkably increased in the musculature of AGF-transgenic mice. AGF-transgenic mice showed a significant decrease in serum leptin levels with a significant decrease in fat mass compared with controls, but showed no changes in serum adiponectin levels, and had increased insulin sensitivity.

AGF as a potential target for developing pharmacological interventions to counteract obesity and related insulin resistance. Plasma triglycerides levels do not appear to be influenced by ANGPTL6 overexpression or deletion, suggesting that ANGPTL6, in contrast to ANGPTL3 and ANGPTL4, does not inhibit LPL activity.

ANGPTL4 expression is under nutritional control: its plasma concentration is increased by fasting and is decreased by high-fat feeding. Serum levels of AGF display up-regulation in conditions such as metabolic syndrome, preeclampsia and diabetes. On the other hand, AGF levels are decreased in patients with renal disease. Women show higher AGF levels than men. Among the components of metabolic syndrome, subjects with high waist circumference or decreased high-density lipoprotein cholesterol had the highest increase of serum AGF.

A SANDWICH ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF HUMAN ANGPTL6

BioVendor Human Angiopoietin-Like Protein 6 ELISA (RAG001R)

Intended use

Human ANGPTL6 ELISA is to be used for the in vitro quantitative determination of human ANGPTL6 in serum, plasma and cell culture supernatant.

- The total assay time is less than 4 hours
- The kit measures total ANGPTL6 in serum, plasma and cell culture supernatant
- Assay format is 96 wells
- Components of the kit are provided ready to use, concentrated or lyophilized

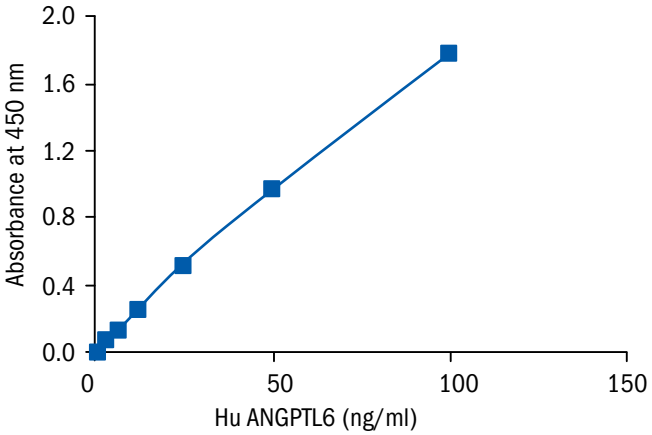
Clinical application

- Renal disease
- Regulation and NO metabolism

Test principle

This assay is a sandwich Enzyme Linked-Immunosorbent Assay (ELISA) for quantitative determination of human ANGPTL6 in biological fluids. A monoclonal antibody specific for ANGPTL6 has been precoated onto the 96-well microtiter plate. Standards an samples are pipetted into the wells for binding to the coated antibody. After extensive washing to remove unbound compounds, ANGPTL6 is recognized by the addition of a purified polyclonal antibody specific for ANGPTL6 (Detection Antibody). After removal of excess polyclonal antibody, HRP conjugated anti-rabbit IgG (Detector) is added. Following a final washing, peroxidase activity is quantified using the substrate 3,3',5,5'-tetramethylbenzidine (TMB). The intensity of the color reaction is measured at 450 nm after acidification and is directly proportional to the concentration of ANGPTL6 in the samples.

HUMAN ANGIOPOIETIN-LIKE PROTEIN 6 ELISA CAT. NO.: RAG001R	
Assay format	Sandwich ELISA, HRP-labelled antibody, 96 wells/kit
Samples	Serum, Plasma-heparin, Plasma-citrate, Plasma-EDTA, Cell culture supernatant
Control	QC
Standards	1.56-100 ng/ml
Limit of detection	Analytical Limit of Detection is calculated from the real human ANGPTL6 values in wells and is 1.2 ng/ml.



A SANDWICH ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF HUMAN ANGPTL6

Precision

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

Six samples of known concentrations of human ANGPTL6 were assayed in replicates 8 times to test precision within an assay.

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	178.45	5.88	3.30
2	662.79	11.25	1.70
3	584.63	9.88	1.69
4	397.97	12.92	3.25
5	739.63	13.15	1.78
6	546.35	8.27	1.51

Inter-assay (Run-to-Run) (n=8)

Six samples of known concentrations of human ANGPTL6 were assayed in 8 separate assays to test precision between assays.

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	179.27	6.03	3.36
2	610.74	52.20	8.55
3	245.16	10.44	4.26
4	747.29	48.71	6.52
5	397.97	12.92	3.25
6	561.28	45.45	8.10

Spiking recovery

When samples (serum) are spiked with known concentrations of human ANGPTL6, the recovery averages 96% (range from 85% to 105%).

Sample	Average recovery (%)	Range (%)
1	88.77	85-95
2	101.17	95-105
3	100.43	95-105

Linearity

Different human serum samples containing ANGPTL6 were diluted several fold (1/10 to 1/40) and the measured recoveries ranged from 95% to 102%.

Sample	Dilution	Observed (ng/ml)	Expected (ng/ml)	% of Expected
1	1 : 10	756.83	756.83	100
	1 : 20	362.26	378.42	95.73
	1 : 40	190.49	189.21	100.68
2	1 : 10	499.35	499.35	100
	1 : 20	245.52	249.67	98.34
	1 : 40	125.66	124.84	100.66
3	1 : 10	888.90	888.90	100
	1 : 20	448.17	444.45	100.84
	1 : 40	220.54	222.23	99.24

Summary of protocol

- Prepare reagents, samples and Standards as instructed
- Add 100 µl Standards, QC and samples
- Incubate for 1 hour at 37°C
- Wash plate 3 times
- Add 100 µl Detection Antibody
- Incubate for 1 hour at 37°C
- Wash plate 3 times
- Add 100 µl diluted Detector
- Incubate for 1 hour at 37°C
- Wash plate 5 times
- Add 100 µl mixed Substrate Solution
- Incubate at RT for 30 min in the dark
- Add 100 µl Stop Solution
- Read absorbance and calculate results within 30 min

Related products

- RD191092200R Human Angiopoietin-Like Protein 3 ELISA
- RD191073200R Human Angiopoietin-Like Protein 4 ELISA

Contact Information

BioVendor – Laboratorni medicina 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

BioVendor GesmbH
Gaudenzdorfer Gürtel 43-45, 1120 Vienna, Austria
Phone: +43 1 890 9025, Fax: +43 1 890 5163
E-mail: infoAustria@biovendor.com

BioVendor GmbH
Otto-Hahn-Straße 16, 34123 Kassel, Germany
Phone: +49 6221 4339 100, Fax: +49 6221 4339 111
E-mail: infoEU@biovendor.com

BioVendor, LLC
128 Bingham Rd., Suite 1300, Asheville, NC 28806, USA
Phone: +1-800-404-7807, Phone: +1-828-575-9250
Fax: +1-828-575-9251, E-mail: infoUSA@biovendor.com

› *www.biovendor.com*

*Visit **www.biovendor.com** to find more information about BioVendor products.*

Date of issue November 2013