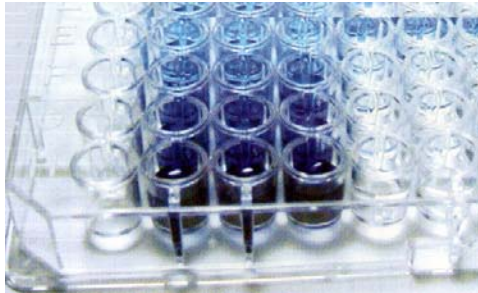


Human ANGPTL3 ELISA Kit

Cat. No. A0822EK



Instruction Manual
Version 1.0.0

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES



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Introduction

The angiopoietins are a family of growth factors that are specific for vascular endothelium. Conklin et al. (1) isolated a full-length cDNA encoding angiopoietin-like protein 3 (ANGPTL3) from a human fetal liver/spleen cDNA library. The deduced 460-amino acid ANGPTL3 protein has the characteristic structure of angiopoietins: a signal peptide, an extended helical domain predicted to form dimeric or trimeric coiled-coils, a short linker peptide, and a globular fibrinogen-like domain (FLD). Human ANGPTL3 shares 76% amino acid sequence identity with mouse Angptl3. Northern blot analysis of human tissues showed a preferential expression of 4 ANGPTL3 transcripts being 4.5, 3.0, 2.8, and 1.7 kb in liver. Camenisch et al. (2) determined showed that ANGPTL3 induced angiogenesis in the rat corneal assay. The FLD alone was sufficient to induce endothelial cell adhesion and in vivo angiogenesis. By microarray analysis, Zhang et al. (3) showed that mouse hematopoietic stem cell (HSC)-supportive fetal liver CD3-positive cells expressed Angptl2 and Angptl3. Long-term HSC expansion occurred when HSCs were cultured in the presence of Angptl2 and Angptl3 together with saturating levels of other growth factors, concluding that angiopoietin-like proteins can be potent stimulators of ex vivo expansion of HSCs. The KK obese mouse is moderately obese and has abnormally high levels of plasma insulin, glucose, and lipids. Koishi et al. (4) observed a mutant mouse strain named KK/San, which showed a hypolipidemia. By positional cloning, they discovered a genetic locus encoding a unique angiopoietin-like lipoprotein modulator was responsible for such hypolipidemia. It was found to be identical to angiopoietin-like protein-3, encoded by Angptl3, and had a highly conserved counterpart in humans. Overexpression of Angptl3 or intravenous injection of the purified protein in KK/San mice elicited an increase in circulating plasma lipid levels. These data suggested that Angptl3 regulates lipid metabolism in animals. The authors suggested the possibility that genetic variation in ANGPTL3 contributes to atherosclerosis, coronary artery disease, and diabetes mellitus. In vitro analysis of recombinant protein revealed that Angptl3 directly inhibits both endothelial lipase and lipoprotein lipase (LPL) activity (5, 6). Another line of evidence suggests that ANGPTL3 play an important role in regulation of HDL synthesis (7). The implication of ANGPTL3 in a number of metabolic dysfunctions suggests that ANGPTL3 is a novel predictor of these.

Assay Principles

This kit is an enzyme-linked immunosorbent assay (ELISA) for quantitative determination of ANGPTL3 in human serum, plasma or cell culture supernatants.

A monoclonal antibody specific for human ANGPTL3 has been pre-coated onto 96 well microplate. Standards and samples are pipetted into the wells and any ANGPTL3 present is bound by immobilized antibody. Bound ANGPTL3 is captured by purified anti-human ANGPTL3 polyclonal antibody. HRP conjugated anti-rabbit IgG is added. After washing, a substrate solution is added. The colors develop in proportion to the bounded ANGPTL3 quantity. The color development is stopped and the intensity of color is measured.

Kit Components

- 1) Antibody coated 96-well plate, 12 X 8-well strips
- 2) 5x Wash concentrate, 100 ml
- 3) 5x Diluent, 50 ml
- 4) Secondary antibody, 12 ml
- 5) 100x Detector, 150 μ l
- 6) Standard, recombinant human ANGPTL3 expressed by *E. coli* cells, 1 vial, lyophilized
- 7) QC sample = recombinant human ANGPTL3 protein, 1 vial, lyophilized (For actual concentrations of QC sample, see the 'Certificate of analysis' enclosed.)
- 8) Substrate, 12 ml
- 9) Stop solution, 12 ml
- 10) Plate sealer, 3 sealers

Reagents Description

Antibody coated 96-well plate, 12 x 8-well strips, with absorbed monoclonal antibody against human ANGPTL3
5x Wash concentrate, buffered detergent solution, supplied as a 5x concentrate

5x Diluent, for sample and reagent dilution
1x Secondary antibody, polyclonal antibody against human ANGPTL3
100x detector, HRP conjugated rabbit IgG
Standard, 20.0 ng, 1 vial each, recombinant human ANGPTL3, lyophilized
QC sample, 1 vial each, recombinant human ANGPTL3 protein
Substrate, chromogenic reagents
Stop solution, 1M H₃PO₄

Storage of Reagents

Reagent must be stored at 2-8°C when not in use. Reagents must be brought to room temperature before use. Do not expose reagents to temperatures greater than 25°C. Diluted wash solution may be stored at room temperature for up to one month.

Materials Required but not Supplied

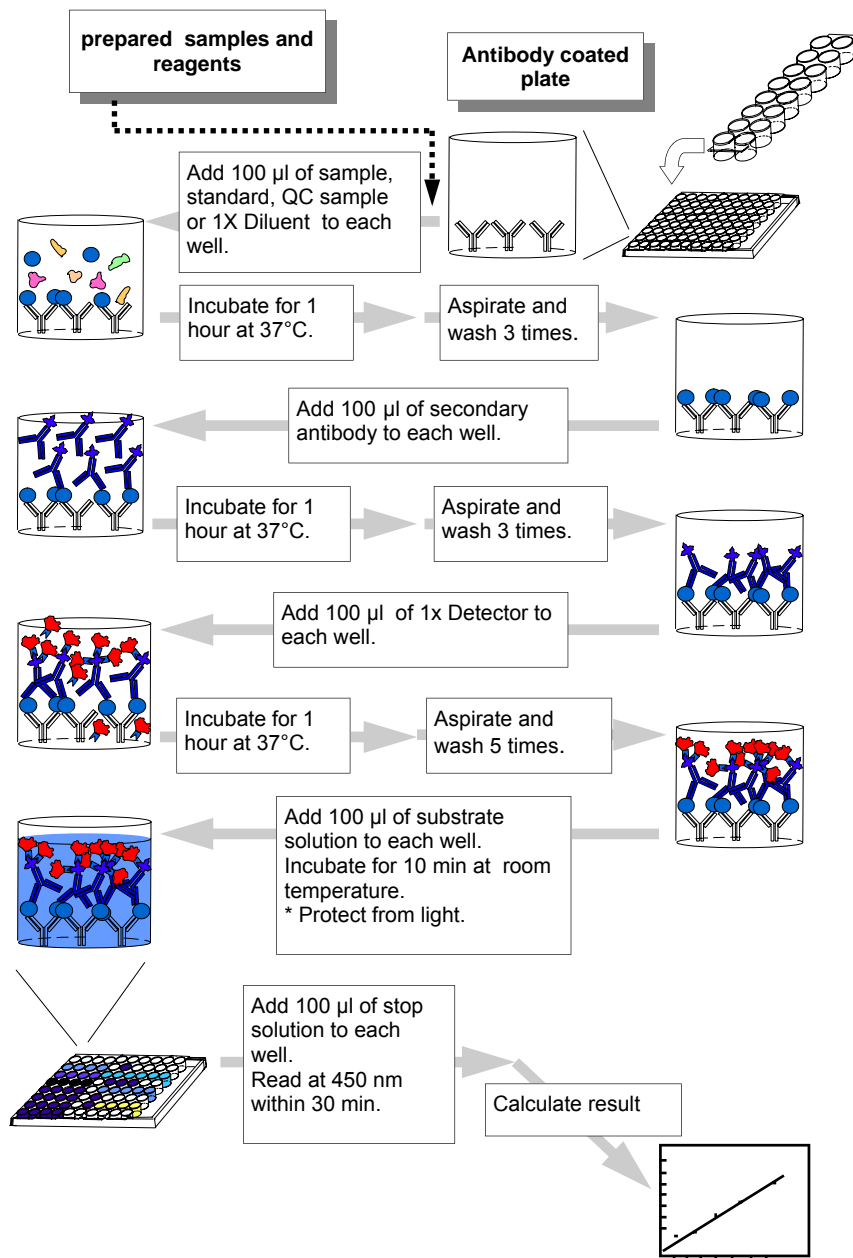
Precision single and multi-channel pipettes
Disposable pipette tips
Microtubes or equivalent for preparing dilutions
Disposable plastic containers for preparing working reagents
Reagent reservoirs
Microwell or microstrip plate reader 450 nm
Deionized water

Sample Collection and Storage

Serum Use a serum separator tube. Let samples clot at room temperature for 30 minutes before centrifugation for 20 minutes at 1,000xg. Assay freshly prepared serum or store serum in aliquot at ≤ -20°C for later use. Avoid repeated freeze/thaw cycles.

Plasma Collect plasma using heparin, EDTA, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay freshly prepared plasma or store plasma sample in aliquot at ≤ -20°C for later use. Avoid repeated freeze/thaw cycles.

Flow Chart of Assay Procedure



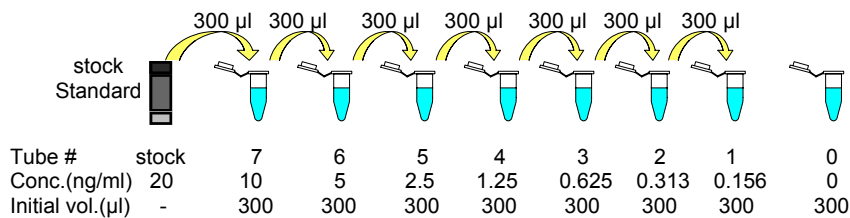
Assay Procedure

1. Preparation of Reagents

- 1) Allow all samples and kit components to equilibrate to room temperature (20-25°C).
- 2) Plan the plate configuration and create a plate map. Calculate the amount of working reagents to use.
It is recommended that Standards and samples be run in duplicate.
- 3) Prepare **1X Wash Solution**. Dilute 5X Wash Concentrate 1:5 with deionized water (1 part 5X Wash Concentrate with 4 parts deionized water). The diluted 1X Wash Solution is stable for one month at room temperature.
- 4) Prepare **1X Diluent**. Dilute 5X Diluent 1:5 with deionized water (1 part 5X Diluent with 4 parts deionized water).
- 5) Prepare **1X Detector**. Dilute 100X Detector 1:100 with 1X Diluent (1 part 100X Detector with 99 parts 1X Diluent). Use the 1X Detector within one hour of preparation.
- 6) Warm **Substrate Solution** to room temperature before use.
- 7) Prepare working aliquots of the **Standard** as follows :
When opening the lyophilized Standard, remove cap gently as the lyophilizate may have become dislodged during shipping.
Add 1 ml of deionized water to the Standard vial to make a stock concentration of 20 ng/ml. Mix well.

A recommended dilution scheme is as follows :

- A) Label 8 microcentrifuge tubes #0-7. Add 300 μ l of the 1X Diluent to the microcentrifuge tubes # 0-7.
- B) Add 300 μ l of the stock Standard solution to tube # 7 and vortex. This is Standard tube # 7 with a concentration of 10 ng/ml.
- C) Standards # 6 to # 1 are then prepared by performing a 1:2 dilution of the preceding standard. Do not add any standard to the tube # 0.



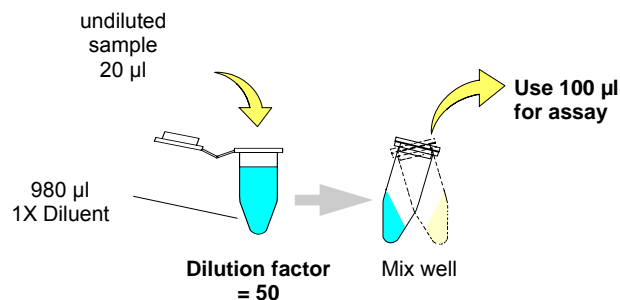
8) Reconstitute QC sample in 1 ml of deionized water. Mix well.

2. Sample Preparation

1) Dilute samples 1:50 with 1X Diluent (example, 20 μ l sample plus 980 μ l 1X Diluent; dilution factor=50) and mix well.

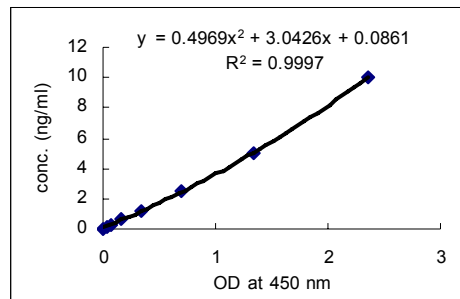
* If samples fall the outside range of assay, a lower or higher dilution may be required.

2) Use 100 μ l of the final diluted sample for ELISA.



3. Experiment procedure

- 1) Remove the appropriate number of microwell strips from the sealed foil pouch.
- 2) Pipette 100 μ l of standard # 0 to # 7, the reconstituted QC sample and diluted sample into the antibody-coated plate according to the plate configuration. Use a new pipette tip for each standard or sample.
- 3) Incubate at 37°C for 1 hour.
- 4) Remove the solution and wash 3 times with 300 μ l of 1X Wash Solution to each well.
- 5) Add 100 μ l Secondary Antibody to each well.
- 6) Incubate at 37°C for 1 hour.
- 7) Remove the solution and wash 3 times with 300 μ l of 1X Wash Solution to each well.
- 8) Add 100 μ l 1X Detector to each well.
- 9) Incubate at 37°C for 1 hour.
- 10) Remove the solution and wash 5 times with 300 μ l of 1X Wash Solution to each well.
- 11) Add 100 μ l of the Substrate Solution to each well.
- 12) Incubate at room temperature for 10 min.
* Protect from light.
- 13) Using the multi-channel pipette, add 100 μ l Stop Solution to each well.
- 14) Read at 450 nm.
- 15) Subtract the absorbance of the blank from the readings for each standard and sample.
- 16) Construct a standard curve by plotting the known concentrations (Y) of standard versus the absorbances (X) of standard. A measurable range is typically shown between 0.156 ng/ml and 10 ng/ml.
- 17) Calculate the ANGPTL3 concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation
- 18) The ANGPTL3 concentrations calculated must be multiplied by dilution factor [see 2. **Sample Preparation**] to obtain the concentrations of the undiluted samples.



Performance Characteristics

1) Sensitivity : 150 pg/ml

2) Precision

a. Intra-Assay (precision within an assay)

6 samples were tested 6 times to assess intra-assay precision.

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	104.10	5.99	5.75
2	44.06	1.55	3.52
3	58.33	1.18	2.02
4	155.19	5.80	3.74
5	61.35	3.63	5.92
6	41.95	2.61	6.21

b. Inter-Assay (precision between assays)

6 samples were tested 8 times to assess inter-assay precision.

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	101.83	8.13	7.98
2	43.75	1.64	3.75
3	56.64	3.55	6.26
4	151.94	11.47	7.55
5	42.51	2.80	6.59
6	71.28	4.59	6.44

3) Specificity

- a. No cross reaction with mouse and rat sera
- b. Cross Reactivity

Analyte	Max. Conc. (ng/ml)	Cross Reactivity (%)
Human ANGPTL3	1	100
Human ANGPTL4	10	N. R.
Human ANGPTL6	10	N. R.
Human ANGPTL7	10	N. R.
Human ANGPTL2	10	N. R.
Human ANG1	10	N. R.
Human ANG2	10	N. R.
Human Adiponectin	10	N. R.
Human Resistin	10	N. R.
Human Vaspin	10	N. R.
Human GPX3	10	N. R.
Human Clusterin	10	N. R.
Human IL-33	10	N. R.
Human FABP4	10	N. R.
Human Leptin	10	N. R.
Human RBP4	10	N. R.
Mouse ANGPTL3	10	N. R.

N. R. : No Cross-reactivity

4) Recovery

The recovery of ANGPTL3 spiked to three different levels in three different human serum samples throughout the range of assay was evaluated.

Sample No.	Average recovery (%)	Range (%)
1	97.99	95-105
2	99.49	95-105
3	106.26	100-110

5) Linearity - Effect of Serum Dilution

Three serum samples were pre-treated as described in the protocol, resulting in the final dilution of x50 (labeled in the table below as dilution; 1).

Sample No.	Serum Dilution	Expected (ng/ml)	Observed (ng/ml)	% Of Expected
1	1	54.19	54.19	100
	1/2	27.09	25.45	93.95
	1/4	13.55	12.59	92.95
2	1	86.58	86.58	100
	1/2	43.29	42.25	97.59
	1/4	21.65	22.13	102.23
3	1	74.66	74.66	100
	1/2	37.33	35.73	95.70
	1/4	18.67	18.83	100.85

$$\% \text{ of expected} = \text{observed} / \text{expected} \times 100\%$$

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Troubleshooting Guide

Problem	Possible Cause	Solution
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be appropriate for the system.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of detector too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.
	Technique problem	Proper mixing of reagents and wash steps are critical.

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