

Human Angiopoietin-like Protein 4 ELISA

Cat. No.: BA3009

Enzyme Immunoassay for the quantitative determination of Angiopoietin-like Protein 4 (ANGPTL-4) in human serum and plasma.

Angiopoietin-like Protein 4 (ANGPTL-4) is a glycoprotein structurally belonging to the angiopoietin/Angptl family and is ubiquitously expressed in human tissues. The highest expression levels in humans are found in liver, followed by the small intestine, adipose tissue, and heart. Angptl4 is strongly induced by fasting in white adipose tissue and liver and has a role in the regulation of lipid metabolism, particularly as an inhibitor of lipoprotein lipase activity. Angptl4 has been implicated in the development of hypertriglyceridemia.

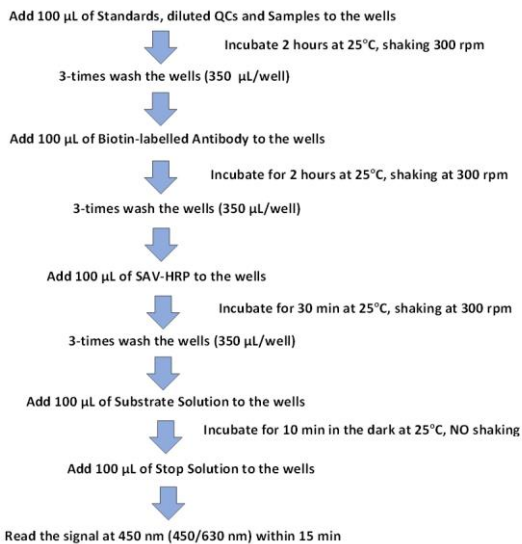
PRINCIPLE OF ANGPTL-4 ELISA

The microtiter plate is coated with the antibody specifically binding the Angiopoietin-like Protein 4. The human serum or plasma (citrate, EDTA) is incubated in the plate with the capture antibody.

The specimen is washed out and the specifically bound protein is incubated with biotin-labelled detection antibody. Following another washing step, Streptavidin-HRP conjugate is added into the well.

Unbound reagent is then washed out. Horseradish peroxidase (HRP) bound in the complex reacts with the chromogenic substrate (TMB) creating the blue colour. The reaction is stopped by addition of STOP solution (H₂SO₄).

The absorbance values are measured at 450 nm (optionally 450/630 nm) and are proportional to the concentration of ANGPTL-4 in the specimen. The concentration of ANGPTL-4 in unknown samples is determined from the calibration curve which is created by plotting the absorbance values against the standard concentration values.



Kit Contents

Item	Qty.
Antibody Coated Microtiter Plate	96 wells
Biotin-labelled Antibody	13 mL
Streptavidin-HRP Conjugate	13 mL
Master Standard (lyophilized)	1 vial
Quality Control A (human serum, lyophilized)	1 vial
Quality Control B (human serum, lyophilized)	1 vial
Dilution Buffer	2x13 mL
Wash Buffer 15x conc.	50 mL
Substrate Solution	13 mL
STOP Solution	13 mL

MATERIAL REQUIRED BUT NOT SUPPLIED

1. Glassware and test tubes
2. Microtiter plate washer
3. Precision pipettes (various volumes) with tips
4. Orbital shaker
5. Microtiter plate reader capable of measuring absorbance at 450 nm or 450/630 nm with software for data generation

WARNINGS AND PRECAUTIONS

1. For research use only
2. For professional laboratory use
3. The reagents with different lot numbers should not be mixed
4. To prevent cross sample contamination, use disposable labware and pipette tips
5. To protect laboratory staff, wear protective gloves and protective clothing
6. The substrate solution should remain colourless, keep it protected from light
7. The test should be performed at standard laboratory conditions (temperature 25°C ± 2°C).

STORAGE CONDITIONS

1. The kit must be stored at 2 – 8°C.
2. The opened components can be stored for one week at 2 – 8°C.

PREPARATION OF REAGENTS

- Use new pipette tip for pipetting different reagents and samples to prevent cross-contamination.
- All reagents and samples should be allowed to reach the temperature 25°C ± 2°C.

Preparation of Standards

Reconstitute lyophilized Human ANGPTL-4 Standard in Dilution Buffer, for the volume information see the Certificate of Analysis. Let it rehydrate for 15 min prior to use. The concentration of human ANGPTL-4 in Master Standard is 60 ng/mL.

Prepare set of Standard solution as follows:

Use the Master Standard for serial dilution (as below). Mix each tube thoroughly before the next transfer. The Dilution Buffer serves as Blank.

	Volume of Standard	Dilution Buffer	Concentration
Std1	Standard 60 ng/mL (lyophilized)	See CofA	60 ng/mL
Std2	300 µL of Std1	300 µL	30 ng/mL
Std3	300 µL of Std2	300 µL	15 ng/mL
Std4	300 µL of Std3	300 µL	7.5 ng/mL
Std5	300 µL of Std4	300 µL	3.75 ng/mL
Std6	300 µL of Std5	300 µL	1.88 ng/mL
Std7	300 µL of Std6	300 µL	0.94 ng/mL
Blank	-	300 µL	0 ng/mL

Preparation of Quality Control A and B

Reconstitute the lyophilized human serum Quality Controls with deionized/distilled water, for the volume information see the Certificate of Analysis. Let the QCs rehydrate for 15 min prior to use.

Preparation of Wash Buffer 1x

Prepare a working solution of Wash Buffer by adding 50 mL of Wash Buffer 15x conc. to 700 mL of deionized/ distilled water (dH₂O). Mix well. Store at 4°C for two weeks or at -20°C for long term storage.

Preparation of samples

Human serum or plasma (citrate, EDTA) may be used with this assay. For long-term storage the samples should be frozen at minimum -70°C. Lipemic or haemolytic samples may cause false results.

Recommended dilution of samples is 1:5, i.e., for singlets 30 µL of sample + 120 µL of Dilution Buffer, for duplicates 60 µL of samples + 240 µL of Dilution Buffer, respectively.

Do not store the diluted samples.

ASSAY PROCEDURE

1. Prepare the reagents as described in the previous chapter.
2. Pipette 100 µL of set of Standards, Quality Controls, diluted Samples and Dilution Buffer = Blank into each well. Incubate for **2 hours** at 25°C ±2°C, shaking at 300 rpm.
3. Wash the wells 3-times with 1x Wash Buffer (350 µL/well). When finished, tap the plate against the paper towel to remove the liquid completely.
4. Pipette 100 µL of Biotin-labelled Antibody into each well. Incubate for **2 hours** at 25°C ±2°C, shaking at 300 rpm.
5. Wash the wells as described in point 3.
6. Pipette 100 µL of Streptavidin-HRP into each well. Incubate for **30 min** at 25°C ±2°C, shaking at 300 rpm.
7. Wash the wells as described in point 3.
8. Pipette 100 µL Substrate solution, incubate for **10 min**, at 25°C ±2°C. Avoid exposure to the light during this step.
9. Pipette 100 µL of STOP solution.
10. Read the signal at 450 or 450/630 nm within 15 min.

PERFORMANCE CHARACTERISTICS

Samples used in the tests were diluted 1:5 as recommended and assayed. The results are multiplied by the dilution factor.

1. Sensitivity

The limit of detection, defined as a concentration of human ANGPTL-4 giving absorbance higher than absorbance of blank + 3 standard deviations, is better than 0.173 ng/mL of sample.

2. Precision

Intra-assay

Sample	Mean (ng/mL)	SD	CV (%)
1	107.3	3.8	3.6
2	24.9	1.0	4.1

Inter-assay (Run – to – run)

Sample	Mean (ng/mL)	SD	CV (%)
1	35.0	2.3	6.7
2	131.4	6.8	5.2

3. Accuracy

Dilution linearity

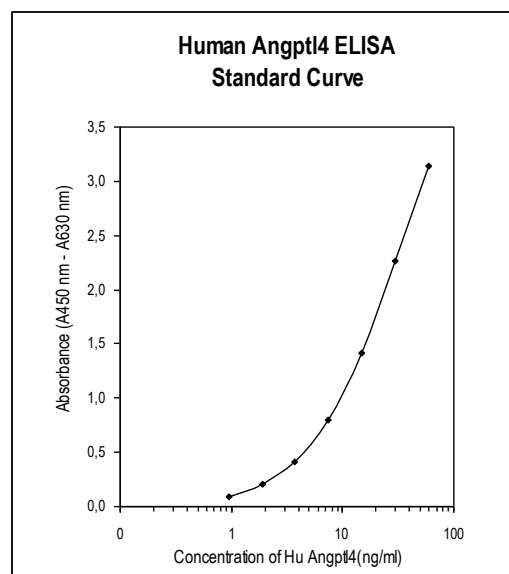
Sample	Dilution	Measured concentration (ng/mL)	Expected concentration (ng/mL)	Yield (%)
1		128.1	-	-
	2x	74.4	64.0	116
	4x	32.8	32.0	102
2		142.9	-	-
	2x	82.6	71.4	116
	4x	39.1	35.7	109

Spiking Recovery

Sample	Spike (ng/ml)	Measured concentration (ng/mL)	Expected concentration (ng/mL)	Yield (%)
1	-	40.7	-	-
	75	100.6	115.7	87
	37.5	71.0	78.2	91
	18.75	60.3	59.5	101

Typical standard curve

The standard curve needs to be measured in every test. Most of the microplate reader can automatically calculate the analyte concentration using 4-parameter algorithm or alternative functions to fit the standard points properly. The concentrations need to be multiplied by the dilution factor, either automatically by reader or manually.



RESOURCES

¹Sukonina V, Lookene A, Olivecrona T, Olivecrona G.: Angiopoietin-like protein 4 converts lipoprotein lipase to inactive monomers and modulates lipase activity in adipose tissue. PNAS November 14, 2006 vol. 103 no. 46 17450-17455

²Xu A., Lam M. C., Chan K. W., Wang Y., Zhang J., Hoo R. L., Xu J. Y., Chen B., Chow W. S., Tso A. W., Lam K. S. (2005): Angiopoietin-like protein 4 decreases blood glucose and improves glucose tolerance but induces hyperlipidemia and hepatic steatosis in mice. Proc. Natl. Acad. Sci. U.S.A. 102, 6086–6091

³Stejskal D, Karpisek M, Reutová H, Humenanská V, Petzel M, Kusnierová P, Varka I, Vareková R, Stejskal P. Angiopoietin-like protein 4: development, analytical characterization, and clinical testing of a new ELISA. Gen Physiol Biophys. 2008 Mar;27(1):59-63.