

Code No. 27749

Human ANGPTL4 Assay Kit - IBL**INTRODUCTION**

Angiopoetin-like 4 (ANGPTL4) is a member of the angiopoietin/ANGPTL family. Human ANGPTL4 consists of 406 amino acids. Its protein structure is common to the angiopoietins, with a signal peptide directing secretion, an N-terminal coiled-coil domain (CCD), a linker, and a C-terminal fibrinogen-like domain (FLD). Full-length ANGPTL4 is secreted by liver and adipose tissues and cleaved to generate circulating CCD and FLD fragments. ANGPTL4 plays important roles in lipid and glucose metabolism. It inhibits lipoprotein lipase (LPL) activity by breaking the dimmer molecule, and elevates plasma TG level. Meanwhile, it is also implicated in breast cancer metastasis to lung via the regulation of vascular integrity.

Both of two antibodies used in this ELISA kit recognize the N-terminal portion of human ANGPTL4 respectively.

PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of highly specific antibodies. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The strength of coloring is proportional to the quantities of Human ANGPTL4.

MEASUREMENT RANGE

23.44 – 1,500 pg/mL

INTENDED USE

This IBL's assay kit is capable for the quantitative determination human ANGPTL4 in serum and EDTA-plasma.

- In the case of normal individuals, many of blood samples will fall below the sensitivity.
- When cell culture media is measured, some suitable arrangements are required (for example, negative media control) since some FBS (FCS) will be measured high level ANGPTL4 with this kit.

KIT COMPONENT

- 1 Precoated plate : Anti-Human ANGPTL4 (K3-21A1A) Mouse IgG MoAb Affinity Purify 96Well x 1
- 2 Labeled antibody Conc. : (30X) HRP conjugated Anti- Human ANGPTL4 (K2-19A1A) Mouse IgG Fab' Affinity Purify 0.4mL x 1
- 3 Standard : Recombinant Human full-length ANGPTL4 0.5mL x 2
- 4 EIA buffer 30mL x 1
- 5 Solution for Labeled antibody* 12mL x 1
- 6 Chromogen : TMB solution 15mL x 1
- 7 Stop solution* 12mL x 1
- 8 Wash buffer Conc.* 50mL x 1

OPERATION MANUAL**1. Materials needed but not supplied**

- Plate reader (450nm)
- Graduated cylinder and beaker
- Refrigerator (as 4°C)
- Paper towel
- Incubator (37°C ± 1°C)
- Washing bottle for precoated plate
- Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"
- Micropipette and tip
- Deionized water
- Graph paper (log/log)
- Tube for dilution of Standard

2. Preparation

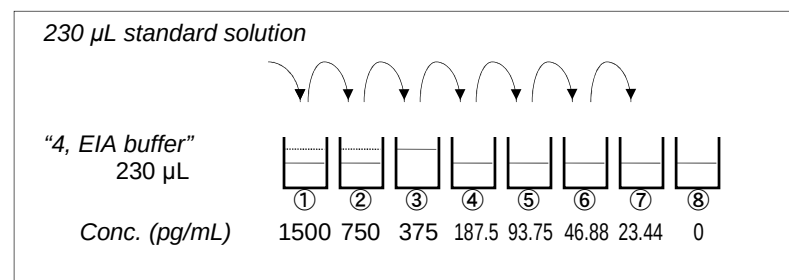
- 1) Preparation of wash buffer
"8, Wash buffer Conc." is a concentrated (40X) buffer. Adjust the temperature of "8, Washing buffer Conc." to room temperature and then, mix it gently and completely before use. Dilute 50 mL of "8, Wash buffer Conc." with 1,950 mL of deionized water and mix it. This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.
- 2) Preparation of Labeled antibody
"2, Labeled antibody Conc." is a concentrated (30X). Dilute "2, Labeled antibody Conc." with "5, Solution for Labeled antibody" in 30 times according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody.
Example)
In case you use one strip (8 well), the required quantity of Labeled antibody is 800 µL. (Dilute 30 µL of "2, Labeled antibody Conc." with 870 µL of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100 µL in each well.)
This operation should be done just before the application of Labeled antibody.
The remaining "2, Labeled antibody Conc." should be stored at 4°C in firmly sealed vial.
- 3) Preparation of Standard
Put just 0.5 mL of deionized water into the vial of "3, Standard" and mix it gently and completely. This solution is 3,000 pg/mL Human ANGPTL4 standard.
- 4) Dilution of Standard
Prepare 8 tubes for dilution of "3, Standard". Put 230 µL each of "4, EIA buffer" into the tube.
Specify the following concentration of each tube."

Tube-1	1,500 pg/mL
Tube-2	750 pg/mL
Tube-3	375 pg/mL
Tube-4	187.5 pg/mL
Tube-5	93.75 pg/mL
Tube-6	46.88 pg/mL
Tube-7	23.44 pg/mL
Tube-8	0 pg/mL (Test Sample Blank)

Put 230 µL of Standard solution into tube-1 and mix it gently. Then, put 230 µL of

tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 1,500 pg/mL and 23.44 pg/mL. Tube-8 is the test sample blank as 0 pg/mL.

See following picture.

**5) Dilution of test sample**

Test samples need to be diluted with "4, EIA buffer" accordingly.

3. Measurement procedure

All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Make sure of no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples.

	Test Sample	Standard	Test Sample Blank	Reagent Blank
Reagents	Test sample 100 µL	Diluted standard (Tube 1-7) 100 µL	EIA buffer (Tube-8) 100 µL	EIA buffer 100 µL
Incubation for 60 minutes at 37°C with plate lid				
4 times (wash buffer more than 350 µL)*				
Labeled Antibody	100 µL	100 µL	100 µL	-
Incubation for 30 minutes at 4°C with plate lid				
5 times (wash buffer more than 350 µL)*				
Chromogen	100 µL	100 µL	100 µL	100 µL
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 µL	100 µL	100 µL	100 µL
Read the plate at 450nm against a Reagent Blank within 30 minutes after addition of Stop solution.				

- 1) Determine wells for reagent blank. Put 100 µL each of "4, EIA buffer" into the wells.
- 2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100 µL each of test sample blank (tube-8), test sample and dilutions of standard (tube-1-7) into the appropriate wells.
- 3) Incubate the precoated plate for 60 minutes at 37°C after covering it with plate lid.
- 4) Wash the plate with the prepared wash buffer and remove all liquid.*
- 5) Pipette 100 µL of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
- 6) Incubate the precoated plate for 30 minutes at 4°C after covering it with plate lid.
- 7) Wash the plate with the prepared wash buffer and remove all liquid.*
- 8) Take the required quantity of "6, Chromogen" into a disposable test tube. Then, pipette 100 µL from the test tube into the wells. Please do not return the rest of the test tube to "6, Chromogen" bottle to avoid contamination.
- 9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The liquid will turn blue by addition of "6, Chromogen".
- 10) Pipette 100 µL of "7, Stop solution" into the wells. Mix the liquid by tapping the side of precoated plate. The liquid will turn yellow by addition of "7, Stop solution".
- 11) Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the liquid. Then, run the plate reader and conduct measurement at 450 nm against a reagent blank. The measurement shall be done within 30 minutes after addition of "7, Stop solution".

SPECIAL ATTENTION

- 1) Test samples should be measured soon after collection. For the storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
- 2) Test samples need to be diluted with "4, EIA buffer", as the need arises.
- 3) Duplicate measurement of test samples and standard is recommended.
- 4) Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
- 5) Use only wash buffer contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
- 6) Remove the wash buffer completely by tapping the precoated plate on paper towel. Do not wipe wells with paper towel.
- 7) "6, Chromogen" should be stored in the dark due to its sensitivity against light. "6, Chromogen" should be avoided contact with metals.
- 8) Measurement should be done within 30 minutes after addition of "7, Stop solution".

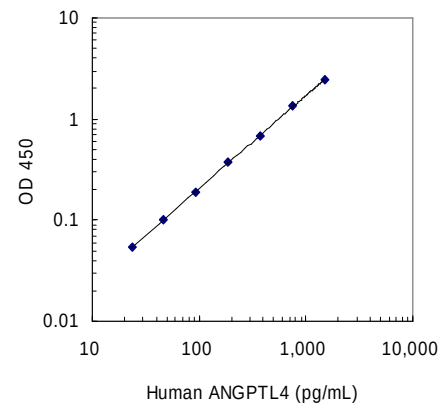
CALCULATION OF TEST RESULT

Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting. Plot the subtracted absorbance of the standards against the standard concentration on log-log graph paper. Draw the best smooth

curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

Example of standard curve

Conc. (pg/mL)	Absorbance (450nm)
1,500	2.480
750	1.364
375	0.705
187.5	0.401
93.8	0.212
46.88	0.125
23.44	0.078
0 (Test Sample Blank)	0.023



* The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

PERFORMANCE CHARACTERISTICS

1. Added Recovery Assay

Specimen	Theoretical Value (pg/mL)	Measurement Value (pg/mL)	%
Human Serum (x2)	509.00	444.57	114.5
	292.79	257.07	113.9
	171.20	163.32	104.8
	120.57	116.44	103.5
Human Plasma (EDTA) (x2)	472.52	442.59	106.8
	266.21	255.09	104.4
	173.84	161.34	107.8
	116.75	114.46	102.0
10%FCS added RPMI-1640 (x4)	1827.04	1573.50	116.1
	1262.89	1198.50	105.4
	1038.31	1011.00	102.7
	927.70	917.25	101.1

2. Intra - Assay

Measurement Value (pg/mL)	SD value	CV value (%)	n
501.49	44.20	8.8	26
146.49	13.02	8.9	26
39.04	4.13	10.6	26

3. Inter - Assay

Measurement Value (pg/mL)	SD value	CV value (%)	n
600.62	57.96	9.7	6
170.88	15.06	8.8	6
39.48	1.93	4.9	6

4. Specificity

Compound	Cross Reactivity
Human ANGPTL4	100 %
Human ANGPTL2	< 0.1 %
Human ANGPTL3	< 0.1 %

5. Sensitivity

17.53 pg/mL

The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.)

PRECAUTION FOR INTENDED USE AND/OR HANDLING

- All reagents should be stored at 2 - 8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
- "3, Standard" is lyophilized products. Be careful to open this vial.
- "7, Stop solution" is a strong acid substance. Therefore, be careful not to have your skin and clothes contact "7, Stop solution" and pay attention to the disposal of "7, Stop solution".
- Dispose used materials after rinsing them with large quantity of water.
- Precipitation may occur in "2, Labeled antibody Conc.", however, there is no problem in the performance.
- Wash hands after handling reagents.
- Do not mix the reagents with the reagents from a different lot or kit.
- Do not use expired reagents.

- This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE AND THE TERM OF VALIDITY

Storage Condition : 2 - 8°C

The expiry date is specified on outer box.

REFERENCE

- Ito Y, Oike Y, Yasunaga K, Hamada K, Miyata K, Matsumoto S, Sugano S, Tanihara H, Masuho Y, Suda T. Inhibition of angiogenesis and vascular leakiness by angiopoietin-related protein 4. *Cancer Res.* 2003 Oct 15;63(20):6651-7.
- Adachi H, Fujiwara Y, Kondo T, Nishikawa T, Ogawa R, Matsumura T, Ishii N, Nagai R, Miyata K, Tabata M, Motoshima H, Furukawa N, Tsuruzoe K, Kawashima J, Takeya M, Yamashita S, Koh GY, Nagy A, Suda T, Oike Y, Araki E. Angptl 4 deficiency improves lipid metabolism, suppresses foam cell formation and protects against atherosclerosis. *Biochem Biophys Res Commun.* 2009 Feb 20;379(4):806-11.

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Made in Japan.