Code No. 27745

# Human ANGPTL2 Assay Kit - IBL

## INTRODUCTION

Angiopoietin-like proteins (ANGPTLs) are secretory form proteins which are similar in structure to angiopoietin which is an angiogenesis factor, and 7 ANGPTLs have been identified. ANGPTL2 has been found to affect vascular cells and monocytes, and it have also been known that ANGPTL3 and ANGPTL4 play important roles in llipid metabolism and AGF (Angiopoietin-like growth factor)/ANGPTL6 plays in energy metabolism. These various biological effects of ANGPTL family are attracting attention as a new target of strategy against lifestyle-related diseases like metabolic syndrome or cancer. There is a report that high level of ANGPTL2 in blood observed in individuals who suffer adipsity, strong insulin resistance, diabetes and arteriosclerosis.
This kit is a quantitative ELISA kit which can measure ANGPTL2 concentration in human serum or plasma.

## 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 ANGPTL2.

## MEASUREMENT RANGE

0.05 - 3.5 ng/mL

## INTENDED USE

**For research use only, not for use in diagnostic procedures.**

- This product is capable for the quantitative determination human ANGPTL2 in serum, EDTA-plasma and cell culture supernatant.
- The recommend dilution for human serum and EDTA-plasma samples is about 10-fold with “4, EIA buffer”.

## KIT COMPONENT

| | | | |
|---|---|---|---|
| 1 | Precoated plate | : | |
| | Anti-Human ANGPTL2 (K2-1A1A) Mouse IgG MoAb Affinity Purify | | 96Well x 1 |
| 2 | Labeled antibody Conc. | : | |
| | (30X) Anti-Human ANGPTL2 (K1-12A4A) Mouse IgG MoAb Fab’ Affinity Purify | | 0.4mL x 1 |
| 3 | Standard | : Human ANGPTL2 | 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)
- Micropipette and tip
- Graduated cylinder and beaker
- Deionized water
- Refrigerator (as 4°C)
- Graph paper (log/log)
- Paper towel
- Tube for dilution of Standard
- Incubator (37°C ± 1°C)
- Washing bottle for precoated plate
- Disposable test tube for “2, Labeled antibody Conc.” and “6, Chromogen”

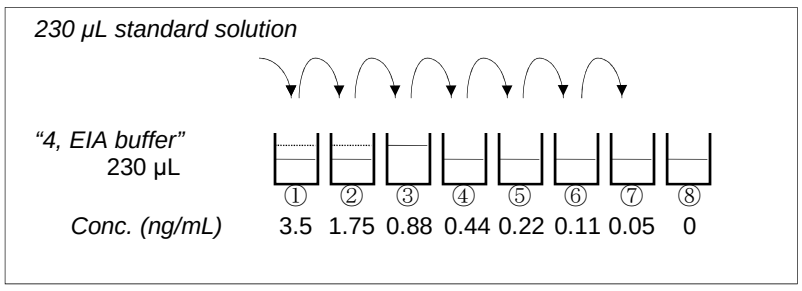
### 2. Preparation

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.)
<u>This operation should be done just before applying Labeled antibody.</u>
The remaining “2, Labeled antibody Conc.” should be stored at 4℃ in firmly sealed vial.
3) Preparation of Standard
Put just <u>0.5 mL</u> of deionized water into the vial of “3, Standard” and mix it gently and completely. This solution is 7 ng/mL Human ANGPTL2 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 | 3.5 ng/mL |
| Tube-2 | 1.75 ng/mL |
| Tube-3 | 0.88 ng/mL |
| Tube-4 | 0.44 ng/mL |
| Tube-5 | 0.22 ng/mL |
| Tube-6 | 0.11 ng/mL |
| Tube-7 | 0.05 ng/mL |
| Tube-8 | 0 ng/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 3.5 ng/mL and 0.05 ng/mL. Tube-8 is the test sample blank as 0 ng/mL.

See following picture.




5) Dilution of test sample
Test samples have to be diluted with “4, EIA buffer” accordingly.
The recommend dilution for human serum and EDTA-plasma samples is about 10-fold.

### 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.

| Reagents | Test Sample | Standard | Test Sample Blank | Reagent Blank |
|---|---|---|---|---|
| | 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” from the bottle into a disposable test tube. Then, pipette 100 µL from the test tube into every well. 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

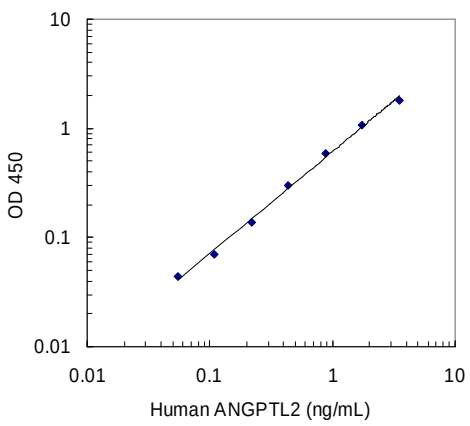
1) Test samples should be measured soon after collection. For the storage of test samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
2) Test samples have to be diluted with “4, EIA buffer”, accordingly.
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*

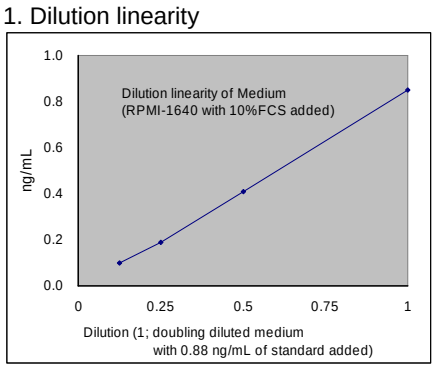
| Conc. (ng/mL) | Absorbance (450nm) |
|---|---|
| 3.5 | 2.923 |
| 1.75 | 1.430 |
| 0.88 | 0.712 |
| 0.44 | 0.376 |
| 0.22 | 0.193 |
| 0.11 | 0.094 |
| 0.05 | 0.050 |
| 0 (Test Sample Blank) | 0.003 |

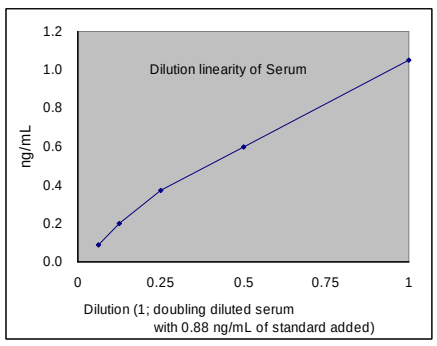


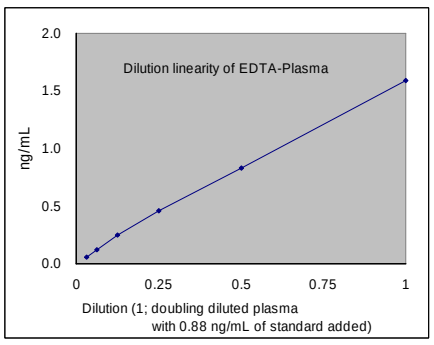

* The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

## PERFORMANCE CHARACTERISTICS

1. Dilution linearity




2. Added Recovery Assay

| Specimen | Theoretical Value (ng/mL) | Measured Value (ng/mL) | % |
|---|---|---|---|
| 10%FCS added RPMI-1640 (x2) | 0.88 | 0.80 | 90.9 |
| | 0.44 | 0.39 | 88.6 |
| | 0.22 | 0.19 | 86.4 |
| Human Serum (x8) | 1.05 | 0.88 | 83.8 |
| | 0.61 | 0.52 | 85.2 |
| | 0.39 | 0.34 | 87.2 |
| Human Plasma (EDTA) (x8) | 1.21 | 1.14 | 94.2 |
| | 0.77 | 0.69 | 89.6 |
| | 0.55 | 0.48 | 87.3 |

3. Intra - Assay

| Mean Value (ng/mL) | SD (ng/mL) | CV (%) | n |
|---|---|---|---|
| 1.54 | 0.06 | 3.9 | 16 |
| 0.35 | 0.02 | 5.7 | 16 |
| 0.17 | 0.01 | 5.9 | 16 |

4. Inter - Assay

| Mean Value (ng/mL) | SD (ng/mL) | CV (%) | n |
|---|---|---|---|
| 1.56 | 0.11 | 7.1 | 4 |
| 0.38 | 0.04 | 10.5 | 4 |
| 0.16 | 0.01 | 6.3 | 4 |

5. Specificity

| Compound | Cross-Reactivity |
|---|---|
| Human ANGPTL2 | 100 % |
| Human ANGPTL3 | < 0.1% |
| Human ANGPTL4 | < 0.1% |
| Human ANGPTL6 | < 0.1% |

6. Sensitivity

0.01 ng/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

1. All reagents should be stored at 2 - 8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
2. “3, Standard” is lyophilized products. Be careful to open this vial.
3. “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”.
4. Dispose used materials after rinsing them with large quantity of water.
5. Precipitation may occur in “2, Labeled antibody Conc.” or “4, EIA buffer” however, there is no problem in the performance.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from a different lot or kit.
8. Do not use expired reagents.
9. 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

1. Tabata M, Kadomatsu T, Fukuhara S, Miyata K, Ito Y, Endo M, Urano T, Zhu HJ, Tsukano H, Tazume H, Kaikita K, Miyashita K, Iwawaki T, Shimabukuro M, Sakaguchi K, Ito T, Nakagata N, Yamada T, Katagiri H, Kasuga M, Ando Y, Ogawa H, Mochizuki N, Itoh H, Suda T, Oike Y. Angiopoietin-like protein 2 promotes chronic adipose tissue inflammation and obesity-related systemic insulin resistance. Cell Metab. 2009 Sep;10(3):178-88.
2. Okada T, Tsukano H, Endo M, Tabata M, Miyata K, Kadomatsu T, Miyashita K, Semba K, Nakamura E, Tsukano M, Mizuta H, Oike Y. Synoviocyte-derived angiopoietin-like protein 2 contributes to synovial chronic inflammation in rheumatoid arthritis. Am J Pathol. 2010 May;176(5):2309-19.
3. Oike Y, Tabata M. Angiopoietin-like proteins--potential therapeutic targets for metabolic syndrome and cardiovascular disease. Circ J. 2009 Dec;73(12):2192-7.

Version 2. November 2016*

Made in Japan.