Trans Genic Inc.

Code No. KK901

FOR RESEARCH USE ONLY

Human Apoptosis Inhibitor of Macrophage (hAIM) ELISA kit

INTRODUCTION

AIM (Apoptosis Inhibitor of Macrophage), a protein produced by macrophage and present in the blood, interacts with both adipose cells and macrophages[1]. By inducing neutral fat breakdown in adipose cells and inhibiting cellular death of macrophage, AIM shows a strong association with various lifestyle-related diseases such as obesity, arteriosclerosis, and diabetes[2-5]. In recent years, it is indicated that AIM plays a role in the pathogenesis of autoimmune disease associated with obesity[6]. As seen above, amount of AIM in the blood is related to pathological condition of many diseases problematic in modern society, and the measurement of AIM is suggested to be effective for diagnostic and therapeutic research of the lifestyle-related diseases. Precise measurement may be difficult depending on the antibody character since AIM binds to IgM in the blood, however the antibody used in this kit is unaffected by IgM, which enables accurate measurement of AIM amount[7].

MEASUREMENT PRINCPLE

This assay kit employs sandwich ELISA method utilizing mouse monoclonal antibody specific for human AIM (hAIM) protein. hAIM-specific antibody is pre-coated on the microplate.

hAIM in standard or specimen (biological sample such as cell culture supernatant and serum) binds specifically to antibody attached on each well of microplate. Then, biotinylated hAIM-specific antibody and HRP-labeled Avidin are added. Finally, colorimetric substrate (TMB) is added and reacted, which produces a color in proportion to the amount of hAIM in the sample. After stopping the enzyme reaction by adding sulfuric acid, the microwell absorbances are measured for the dominant wavelength at 450 nm (reference wavelength: around 630 nm) utilizing microplate reader. Plot the calibration curve using hAIM Standard, and hAIM concentration in the specimen is determined by finding the absorbance value on the calibration curve.

KIT COMPONENT

- Antibody Coated Wells --- 1 (6 strips)
- Biotinylated Antibody (30×) --- 1 (200 µL)
- HRP-labeld Avidin (30×) --- 1 (200 µL)
- Enzyme Diluent --- 1 (12 mL)
- Standard/Sample Diluent --- 1 (30 mL)
- Wash Concentrate (20×) --- 1 (30 mL)
- TMB Substrate Reagent --- 1 (12 mL)
- Stop Solution (sulfuric acid) --- 1 (12 mL)
- hAIM Standard (freeze dried) --- 1 (add 250 µL distilled water just before use)

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measurement at 450 nm [reference wavelength at around 630 nm]
- Precision pipettes or multichannel pipettes to deliver 10 µL -1000 µL volumes, and disposable tips
- Tubes with low-protein adsorbent to prepare standard dilutions
- Measuring cylinder or beaker (500 mL) to prepare wash solution
- Distilled water

OPERATING PRECAUTIONS

- Do not mix reagents from different kit lots.
- Diluted reagents should not be stored for more than 24 hours.
- Do not discontinue the operation during the experimentation. Also, do not leave the microplate dehydrated during operation.
- Use microplate lid to prevent drying during operation if needed.
- All standards and samples are recommended to be run in duplicate.

SPECIMEN PREPARATION

Biological samples such as cell culture supernatant and serum should be diluted with Standard/Sample Diluent.

Recommended dilution ratio of serum is 1/500～1000.

REAGENT PREPARATION

- Biotinylated Antibody solution

Add 180 µL of 30×Biotinylated Antibody to 5220 µL Standard/Sample Diluent to prepare 5.4 mL solution.

- HRP-labeled Avidin solution

Add 180 µL of 30×HRP-labeled Avidin to 5220 µL Standard/Sample Diluent to prepare 5.4 mL solution.

- Wash Buffer

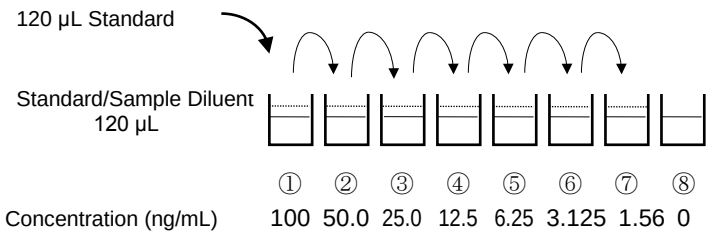
Add 25 mL of Wash Concentrate to 475 mL of distilled water to prepare 500 mL solution.

- hAIM Standard

Reconstitute hAIM Standard with 250 µL of distilled water to prepare a 200 ng/mL stock standard. Add 120 µL Standard/Sample Diluent to 8 tubes, respectively. Perform serial doubling dilutions by adding 120 µL of each standard to the next tube to create concentrations as described below.

#1; 100 ng/mL, #2; 50.0 ng/mL, #3; 25.0 ng/mL, #4; 12.5 ng/mL, #5; 6.25 ng/mL, #6; 3.125 ng/mL, #7; 1.5625 ng/mL, #8; 0 ng/mL

Figure: Serial Dilution of hAIM standard



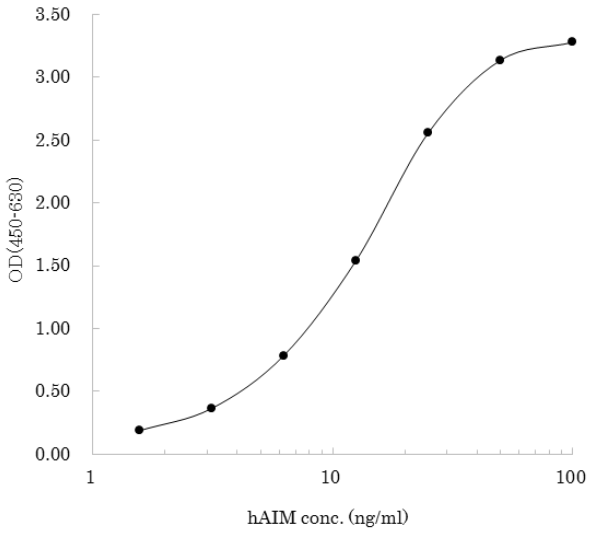
ASSAY PROCEDURE

1. Pipette 300 µL of Wash Buffer to each well, and soak at 25°C for 20 minutes. (See Flowchart indicated below)
2. Wash wells by filling with 300 µL/well Wash buffer, and tap the plate to remove any residual buffer. Repeat wash 3 times.
3. Pipette 50 µL of each standard or sample into antibody coated wells, and incubate for 1 hour at 37°C.
4. Wash wells as in Step 2.
5. Pipette 50 µL of Biotinylated Antibody solution into each well, and incubate for 1 hour at 37°C
6. Wash wells as in Step 2.
7. Add 50 µL of HRP-labeled Avidin solution into each well, and incubate for 1 hour at 37°C.
8. Wash wells as in Step 2.
9. Add 100 µL of TMB Substrate Reagent to each well. Incubate for 10 minutes at room temperature (25°C) in the dark.
10. Add 100 µL of Stop Solution to each well.
11. Read absorbance at 450 nm (reference wavelength at 630 nm) utilizing microplate reader.

Figure: Flowchart

	Test Sample	Standard	Test Sample Blank	Reagent Blank
Reagents	Test sample 50 µL	Diluted Standard (Tube-1～-7) 50 µL	Standard/Sample Diluent (Tube-8) 50 µL	Standard/Sample Diluent 50 µL
Incubation for 1 hour at 37°C with plate lid				
Washing 3 times				
Biotinylated Antibody	50 µL	50 µL	50 µL	—
Incubation for 1 hour at 37°C with plate lid				
Washing 3 times				
HRP-labeled Avidin	50 µL	50 µL	50 µL	—
Incubation for 1 hour at 37°C with plate lid				
Washing 3 times				
Chromogen	100 µL	100 µL	100 µL	100 µL
Incubation for 10 minutes at room temperature (shielded)				
Stop solution	100 µL	100 µL	100 µL	100 µL
Read the plate at ⊿450 nm -630 nm within 30 minutes after application of Stop solution				

STANDARD CURVE



REPRODUCIBILITY

Domain of standard curve：1.56～100 ng/mL
Minimum measurement value for detection：3.13 ng/mL
Minimum dilution rate of the serum sample：500 fold
Within-run (n=10, 3 concentration) : CV(%) =12.4、3.19、3.94
Between-run (n=10, 3 concentration) : CV(%) =12.4、5.07、8.43
Recovery test : In the recovery study, recoveries within the range of 91.8-116.9% were obtained when hAIM in known concentration (30 ng/mL, 7 ng/mL) were added into 500 times dilutions of the sample serum.

STORAGE INFORMATION

Store kit at 2-8°C.

Expiration date is indicated on the outside of the package.

WARNINGS AND PRECAUTIONS

- Store kit at 2-8°C.
- This kit is intended for research use, not for clinical diagnostic use.
- Please handle the reagent with care. Especially, avoid contact of skin, clothing, eyes, or mouth with sulfuric acid or Substrate Reagents.
- Handle biological samples with extreme caution to prevent infection.
- Store kit in defined condition, and use before the expiration date.
- Comply with the local regulations for disposal of used plates, tubes, and waste solution.

REFERENCES

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2) Arai, S. *et al.* (2005) Cell Metab. 1: 201-213.
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4) Kurokawa, J. *et al.* (2011) Proc. Natl. Acad. Sci. USA. 108: 12072-12077.
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6) Arai, S., *et al.* (2013) Cell Rep., 3: 1187-98.
7) Oba, M. *et al*. (2012) Seikagaku 84: 588-591.

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