

## 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<sup>1)</sup>. By inducing neutral fat breakdown in adipose cells and inhibits cellular death of macrophage, AIM shows a strong association with various lifestyle-related diseases such as obesity, arteriosclerosis, and diabetes<sup>2-5)</sup>. In recent years, it is indicated that AIM plays a role in the pathogenesis of autoimmune disease associated with obesity<sup>6)</sup>. 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 is attached to IgM in the blood, however the antibody used in this kit is unaffected by IgM, which enables accurate measurement of AIM<sup>7)</sup>.

### MEASUREMENT PRINCIPLE

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 650 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-labeled 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 250uL distilled water just before use)

**MATERIALS REQUIRED BUT NOT PROVIDED**

- Microplate reader capable of measurement at 450 nm [reference wavelength at around 650 nm]
- Precision pipettes or multichannel pipettes to deliver 10  $\mu$ L -1000  $\mu$ L volumes, and disposable tips
- Tubes with low-protein absorbent 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 200  $\mu$ L of 30 $\times$ Biotinylated Antibody to 5800  $\mu$ L Standard/Sample Diluent to prepare 6.0 mL solution.

- HRP-labeled Avidin solution

Add 200  $\mu$ L of 30 $\times$ HRP-labeled Avidin to 5800  $\mu$ L Standard/Sample Diluent to prepare 6.0 mL solution.

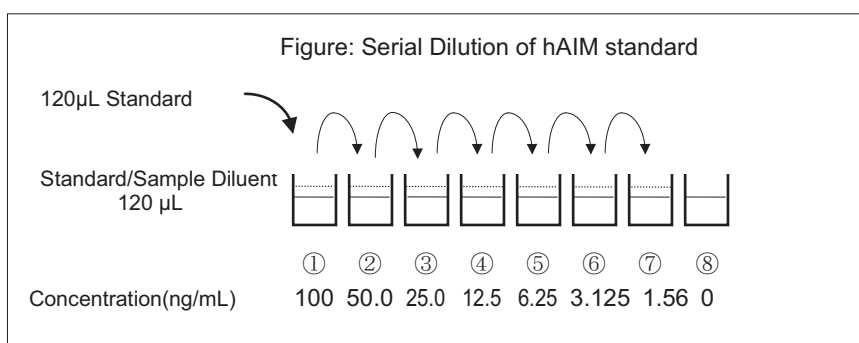
- Wash Buffer

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

- hAIM Standard

Reconstitute hAIM Standard with 250  $\mu$ L of distilled water to prepare a 200 ng/mL stock standard. Add 120  $\mu$ L Standard/Sample Diluent to 8 tubes, respectively. Perform serial doubling dilutions by adding 120  $\mu$ L 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



## ASSAY PROCEDURE

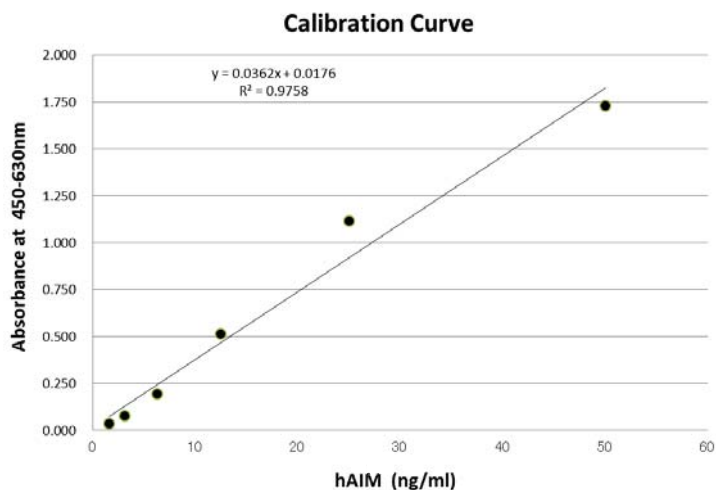
1. Pipette 300 µL of Wash Buffer to each well, and soak at 25°C for 20 minutes. (See Flowchart indicated below)
2. Wash wells by filling with 300 µL/well Wash buffer, and tap the plate to remove any residual buffer. Repeat wash 3 times.
3. Pipette 50 µL of each standard or sample into antibody coated 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 650nm) 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 450nm -650nm within 30 minutes after application of Stop solution				

## CALCULATION OF RESULTS

hAIM concentration may be calculated by utilizing calculation program stored in most of the microplate readers. Also, hAIM concentration in the sample is determined by finding the absorbance value on the calibration curve.



**PERFORMANCE**

Within-run reproducibility (n = 10); CV &lt; 10%

Between-run reproducibility (n = 10); CV &lt; 10%

**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**

- 1) Miyazaki, T. et al (1999) J. Exp. Med 189: 413-422.
- 2) Arai, S. et al. (2005) Cell Metab. 1: 201-213.
- 3) Kurokawa, J. et al. (2010) Cell Metab. 11: 479-492.
- 4) Kurokawa, J. et al. (2011) Proc. Natl. Acad. Sci. USA. 108: 12072-12077.
- 5) Miyazaki, T. et al. (2011) Cir. J. 75: 2522-2531.
- 6) Arai, S., et al. (2013) Cell Rep., 3: 1187-98.
- 7) Oba, M. et al. (2012) Seikagaku 84: 588-591.

**【 Distributor 】****COSMO BIO Co., LTD.**

Inspiration for Life Science

TOYO EKIMAE BLDG. 2-20, TOYO 2-CHOME, KOTO-KU, TOKYO 135-0016 JAPAN

URL: <http://www.cosmobio.com> e-mail: [export@cosmobio.co.jp](mailto:export@cosmobio.co.jp)

Phone: +81-3-5632-9617 FAX: +81-3-5632-9618

**Manufacturer**

7-1-14 Minatojimaminami-machi, Chuo-ku, Kobe, Japan 650-0047

Telephone: +81-78-306-0295 FAX: +81-78-306-0296

URL: <http://www.transgenic.co.jp> techstaff@transgenic.co.jp