Human TIM-1 / KIM-1 ELISA Kit

Enzyme Immunoassay for the quantification of Human TIM-1 / KIM-1 in serum, plasma, cell culture supernatants

Catalog number: ARG81271

distributed in the US/Canada by:
EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com • info@eaglebio.com

For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

SECTION **Page**

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

TIM-1 / KIM-1 is a membrane receptor for both human hepatitis A virus (HHAV) and TIMD4. The encoded protein may be involved in the moderation of asthma and allergic diseases. The reference genome represents an allele that retains a MTTVP amino acid segment that confers protection against atopy in HHAV seropositive individuals. Alternative splicing of this gene results in multiple transcript variants. Related pseudogenes have been identified on chromosomes 4, 12 and 19. [provided by RefSeq, Apr 2015]
TIM-1 / KIM-1 may play a role in T-helper cell development and the regulation of asthma and allergic diseases. Receptor for TIMD4 (By similarity). In case of human hepatitis A virus (HHAV) infection, functions as a cell-surface receptor for the virus. May play a role in kidney injury and repair. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for TIM-1 / KIM-1 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any TIM-1 / KIM-1 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for TIM-1 / KIM-1 is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of TIM-1 / KIM-1 bound in the initial step. The color development is stopped by the addition of acid and the

intensity of the color is measured at a wavelength of 450nm ±2nm.The concentration of TIM-1 / KIM-1 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard (Lyophilized)	2 X 1 ng/vial	4°C
Standard/Sample diluent	1 X 16 ml	4°C
Antibody conjugate concentrate	2 vial (60 µl)	4°C
Antibody diluent buffer	16 ml	4°C
HRP-Streptavidin concentrate	2 vial (60 µl)	4°C (Protect from light)
HRP-Streptavidin diluent buffer	16 ml	4°C
20X Wash buffer	25 ml	4°C
TMB substrate	12 ml	4°C (Protect from light)
STOP solution	12 ml	4°C
Plate sealer	4 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 610-650 nm as the reference wave length)
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation and aliquot & store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma on ice using EDTA or heparin as an anticoagulant. Centrifuge (1000 x g) for 15 minutes at 2-8 °C within 30 minutes of collection.

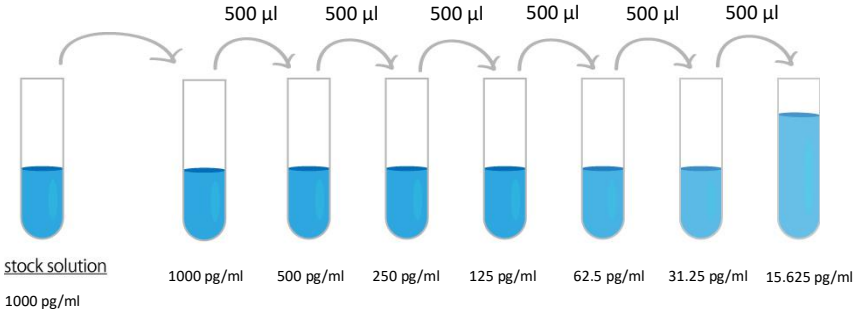
Collect the supernatants and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer.
- 1X Antibody conjugate: Dilute 100X antibody conjugate concentrate into 1X antibody diluent buffer with Antibody diluent buffer to yield 1X detection antibody solution.
- 1X HRP-Streptavidin Solution: Dilute 100X HRP-Streptavidin concentrate solution into 1X HRP-Streptavidin diluent buffer with HRP-Streptavidin diluent buffer to yield 1X HRP-Streptavidin Solution buffer.
- Sample: If the initial assay found samples contain TIM-1 / KIM-1 higher than the highest standard, the samples can be diluted with Standard/Sample diluent and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

  (It is recommended to do pre-test to determine the suitable dilution factor).
- Standards: Reconstitute the standard with 1 ml Standard/Sample diluent to yield a stock concentration of 1000 pg/ml. Allow the stock standard to sit for at least 15 minutes with gentle agitation to make sure the standard is dissolved completely before making serial dilutions. The Standard/ Sample diluent serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with Standard/ Sample diluent as

according to the suggested concentration below: 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.25 pg/ml, 15.625 pg/ml.



ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards, samples and zero controls (Standard/Sample diluent) into wells. Incubate for 1.5 h at 37 °C.
3. Aspirate each well and wash, repeating the process three times for a total four washes. Wash by filling each well with 1× Wash Buffer (350 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add 100 µl 1X Antibody conjugate into each well. Cover wells and incubate

for 1 hour at 37 °C.

5. Aspirate each well and wash as step 3.
6. Add 100 µl of 1X HRP-Streptavidin solution to each well. Cover wells and incubate for 30 minutes at 37 °C.
7. Aspirate each well and wash as step 3.
8. Add 100 µl of TMB Reagent to each well. Incubate for 10-20 minutes at 37°C in dark.
9. Add 100 µl of Stop Solution to each well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough mixing
10. Read the OD with a microplate reader at 450nm immediately. (optional: read at 610-650 nm as the reference wave length)

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

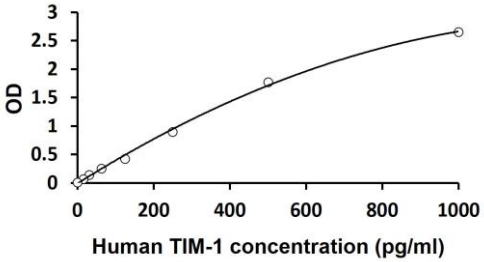
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give

slightly different results.

5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Human TIM-1 / KIM-1 ranged from 15.6- 1000 pg/ml. The mean MDD was 7 pg/ml.

Specificity

This assay recognizes natural and recombinant Human TIM-1 / KIM-1. No significant cross-reactivity or interference with the factors below was observed:

50 ng/ ml of recombinant proteins:

Human: TIM3, TIM4

Mouse: TIM1, TIM4

Rat: TIM1

Intra-assay and Inter-assay precision

The CV values of both intra and inter precision fall below 10%.

distributed in the US/Canada by:

EAGLE BIOSCIENCES, INC.

20A NW Blvd, Suite 112 Nashua, NH 03063

Phone: 617-419-2019 ● FAX: 617-419-1110

www.EagleBio.com ● info@eaglebio.com

For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.