HIGH SENSITIVITY TISSUE TIM-3/HAVCR2 (HUMAN) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN TIM-3 CONCENTRATIONS IN SERUM AND EDTA PLASMA

ALWAYS REFER TO LOT SPECIFIC PROTOCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ AND CHECK ALL ITEMS OF EACH KIT BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PRODUCT INFORMATION:

THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	HIGH SENSITIVITY TIM-3 (HUMAN) ELISA KIT
Catalog No.	SK00775-06
Lot No.	20115158
Formulation	96 T
Standard range	25 - 1600 pg/mL
Sensitivity	7 pg/mL
Calibration:	Human Soluble TIM-3 Rec. (HEK293)
Sample Volume	100 µl
Sample Type	Serum, EDTA Plasma
Dilution Factor	20~ 40 (Optimal dilutions should be determined by each laboratory for each application)
Calibration	Human Soluble TIM-3 (HEK293)
Specificity	Human TIM-3
Intra-assay Precision	4-6%
Inter-assay Precision	6-10%
Storage	2°C - 8°C for 4 months. See page 2-3 for more information.
This kit contains sufficient materials to run approximately 35 – 40 samples duplicated provided that assay is run according to protocol.	

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INTRODUCTION

The High Sensitivity Soluble TIM-3 (Human) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human TIM-3 from serum or Plasma in a sandwich ELISA format.

This immunoassay contains recombinant human TIM-3 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural TIM-3 samples

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal Anti Rabbit IgG Fc has been pre-coated onto a microplate (RM01). A Rabbit Anti human TIM-3 Monoclonal Antibody as capture antibody was added to the Microplate. After capture antibody incubation, wash it. Standards and samples are pipetted into the wells and any human TIM-3 present is bound by the immobilized antibody. After washing away any unbound substances, a HRP conjugated antibody specific for human TIM-3 is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of TIM-3 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_The kit should not be used beyond the expiration date on the kit label.

_Do not mix or substitute reagents with those from other lots or sources.

_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
Anti Rabbit IgG Fc Microplate - 96 well microplate (12 strips of 8 wells) coated with an antibody against Rabbit IgG Fc.	**RM01**	**1 plate**
Capture Antibody Concentrate – 0.9 mL/vial, 10-fold concentrate of rabbit monoclonal antibody against TIM-3 liquid.	**775-06-01**	**1 vial**
TIM-3 Standard – 1600 pg/vial of recombinant human TIM-3; lyophilized.	**775-06-02**	**1 vial**
Detection Antibody HRP Concentrate – 160 µL/vial, 50-fold concentrate of antibody HRP Conjugated against TIM-3 liquid.	**775-06-03**	**1 vial**
Positive Control - one vial of recombinant human TIM-3, lyophilized	**775-06-04**	**1 vial**
Dilution Buffer – 45 mL of buffered protein based solution with preservatives	**DB01**	**1 bottle**
Antibody Diluent Solution – 10 mL of buffered protein based solution with preservative.	**DB0111CM**	**1 bottle**
Wash Buffer 20X - 25mL of 20-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution -11mL of TMB substrate solution	**TMB01**	**1 bottle**
Stop Solution - 11mL of 0.125M HCl	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 4 months. For longer storage up to 10 months, unopened Standard, Positive Control, Capture Antibody Concentrate, Dilution Buffer and Antibody Diluent Solution should be stored at -20° C. Detection

Antibody-HRP Conjugate and TMB Substrate Solution should be stored only at 2-8° C. Do not use kit past expiration date.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- Microplate shaker (200-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

SAMPLE COLLECTION AND STORAGE

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 using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.

Note: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Serum or EDTA plasma samples may require a 20 ~ 40 fold dilution. A suggested 20-fold dilution is 15 µL of sample + 285µL of Dilution Buffer. Add 100 µL per well of 20-fold diluted samples.

A suggested 40-fold dilution is 50 µL per well of 20-fold diluted sample + 50 µL per well of Dilution Buffer.

Optimal dilutions should be determined by each laboratory for each application.

Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute **25 mL of Wash Buffer Concentrate 20X** into deionized or distilled water (**475 mL**) to prepare 500 mL of Wash Buffer.

Prepare Capture Antibody Microplate :

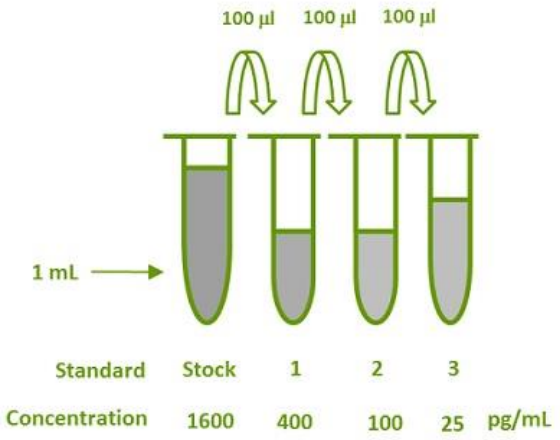
Anti Rabbit IgG Fc Microplate RM01 cannot be directly used. That Microplate RM01 should be incubated with the capture antibody working solution for 90 min at room temperature on a microplate shaker or for overnight at 2 ~ 8 °C.

(Note: Prepare 30 min prior to use)

1. **Capture Antibody Preparation-** For 96 wells test, freshly Pipette 7.2 mL of **Dilution Buffer** into a 15 mL centrifuge tube and transfer 0.8 m of 10-fold concentrated stock solution to prepare capture antibody working solution. For the partial strip test, freshly prepare **700 µL** per strip of the working solution. Add **75 µL** per well of 1x working solution of Capture Antibody.
2. **Capture Antibody Microplate Incubation**: Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack and seal. Use 8-channel Pipette, add 75 µL per well of capture antibody working solution. Cover with plate sealer. Incubate for 90 min on micro-plate shaker at room temperature. Optional incubate it for overnight at 2~ 8 °C without microplate shaker.
3. **Wash Capture Antibody Microplate**: Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **1X Wash Buffer** (275 µL) using a manifold dispenser, or microplate washer. Complete removal of liquid at each step is essential to good performance. Cover it with plate sealer. Store it at 2 ~ 8 °C for next use. Note: Prepare 30~40 min prior to use.

TIM-3 Standard. Reconstitute the **TIM-3 Standard** with 1 mL of Dilution Buffer. This reconstitution produces a stock solution of 1600 pg/mL. Allow the standard to sit for a minimum of 3~5 minutes with gentle agitation prior to making dilutions. Pipette 300 µL of Dilution Buffer into tubes #1 to #3. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **1600 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL). Store the stock solution at -70°C for a few days. Add 100 µL per well of each standard solution or blank.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	Powder	1000 µl	1600 pg/ml
# 1	100 µl of stock	300 µl	400 pg/ml
# 2	100 µl of 1	300 µl	100 pg/ml
# 3	100 µl of 2	300 µl	25 pg/ml



Detection Antibody HRP Conjugated - For 96 wells test, freshly Pipette 7.84 mL of **Antibody Diluent Solution (DB0111CM)** into a 15 mL centrifuge tube and transfer 160µl of 50-fold concentrated stock solution to prepare working solution. For the partial strip test, freshly prepare 700 µL per strip of the working solution. Store the stock solution at 2 ~ 8°C for a few days. Add 75 µL per well of 1x working solution of Detection Antibody HRP Conjugate.

Positive Control - Reconstitute the **Positive Control Concentrate** with 1 **mL** of **Dilution Buffer** to prepare working solution. **Note:** Positive Control should be prepared and used immediately. Discard the positive control after use.

A Capture Antibody Microplate should be prepared and washed 20 ~ 30 min prior to add standard or diluted samples. Refer to page 3.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove the seal from the ***Capture Antibody Microplate***.
3. Add 100 µL of **Dilution Buffer** to Blank wells (F2, F3).
4. Add 100 µL of **Standard** (from B2, B3 to E2, E3), **sample, or positive control** (A2, A3) per well. Cover with plate sealer. Incubate for 2 hours on micro-plate shaker at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **1X Wash Buffer** (300 µL) using a manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 75 µL per well of **Detection Antibody HRP Conjugate working solution** to each well. Cover with plate sealer. Incubate for 90 min on micro-plate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 75 µL of **Substrate Solution** to each well. Incubate for 25 minutes on a micro-plate shaker at room temperature. **Protect from light.**
11. Add 75 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 5~10 minutes, using a micro-plate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log or 4-parameter curve fit.
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human Soluble TIM-3 derived from HEK293.

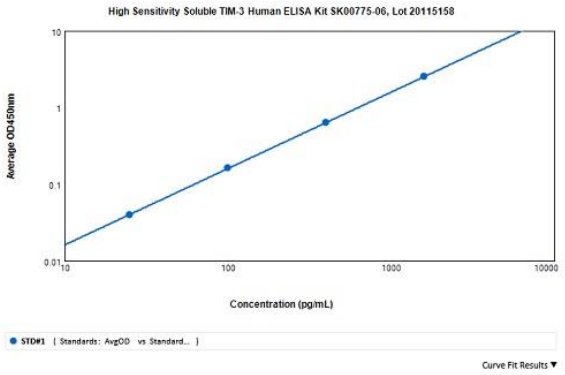
TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Standard (pg/mL)	Average OD450nm (Corrected)
Blank	0 (0.100)
25	0.029
100	0.144
400	0.614
1600	2.557

- Lot No.: 20115158
- Positive Control: 80 – 400 pg/mL (log-log)

Standard curve fit by log-log:



SPECIFICITY

Protein	Cross-reactivity (%)
Human Soluble TIM-3 (HEK293)	100
Mouse Soluble TIM-3 (HEK293)	0

SUMMARY OF ASSAY PROCEDURE

PREPARE CAPTURE ANTIBODY MICROPLATE PRIOR TO USE

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PREPARE REAGENTS, SAMPLES AND STANDARDS

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Add 100 µl of standard, samples, positive control to each well. Incubate 2 hours on the plate shaker at RT.

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Aspirate and wash 4 times.

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Add 75 µl Detection Antibody HRP working solution to each well. Incubate 90 min on the plate shaker at RT.

↓

Aspirate and wash 4 times.

↓

Add 100 µl Substrate Solution to each well. Incubate 25 min on the plate shaker. **Protect from light**.

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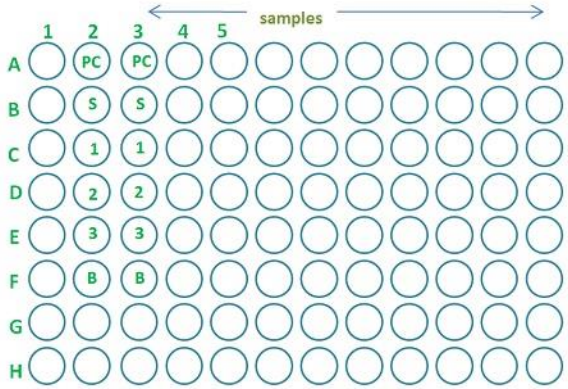
Add 100 µl Stop Solution to each well. Read 450nm within 5 min



The Biomarker ELISA KITS were manufactured by Aviscera Bioscience Research Use Only.

Biomarker Name	Catalog No.
Endotrophin ETP Human ELISA Kit	SK00009-08
Soluble TREM2 Human ELISA Kit	SK00218-12A
Soluble CSF1R Human ELISA Kit	SK00144-01
EPDR1 Human ELISA Kit	SK00023-06
FAM19A1/TAFA1 Human ELISA Kit	SK00419-06
Human Pro-BDNF (19-128) ELISA Kit	SK00752-10
Human Sortilin ELISA Kit	SK00472-01
Human Lipocalin 13 ELISA Kit	SK00648-06
Human METRNL ELISA Kit	SK00478-06