Quantikine™ ELISA

Human Urinary TIM-1/KIM-1/HAVCR Immunoassay

Catalog Number DKM100
SKM100

For the quantitative determination of human T cell Immunoglobulin-Mucin (TIM-1) concentrations in urine.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

SECTION PAGE

Manufactured and Distributed by:

USA R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
TEL: 800 343 7475 612 379 2956
FAX: 612 656 4400
E-MAIL: info@bio-techne.com

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park
Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
TEL: +86 (21) 52380373 (400) 821-3475
FAX: +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

INTRODUCTION

T cell immunoglobulin and mucin domain 1 (TIM-1), also known as Kidney injury molecule 1 (KIM-1) and Hepatitis A virus cellular receptor 1 (HAVcr1), is a member of the TIM family which is involved in the regulation of innate and adaptive immune responses (1, 2). TIM-1 is a type I transmembrane protein that contains an N-terminal immunoglobulin-like domain, a mucin domain with O- and N-linked glycosylations, a transmembrane segment, and a cytoplasmic signaling domain (3, 4). Multiple TIM-1 variants can be produced due to polymorphisms or alternative splicing resulting in deletions in the mucin domain (3). Some of these polymorphisms are associated with susceptibility to atopy, autoimmunity, and severe hepatitis A virus infection in humans (5). Within the extracellular domain, human TIM-1 shares 41% amino acid sequence identity with mouse and rat TIM-1.

In vivo, TIM-1 is expressed on splenic B cells and is a marker for the identification of IL-10$^+$ regulatory B cells (6, 7). TIM-1 is also expressed on CD4$^+$ T cells, mast cells, invariant NKT (iNKT) cells, dendritic cells, kidney epithelium and a broad range of mucosal epithelium (4, 8-15). The expression of TIM-1 is upregulated on activated Th2 cells, after dendritic cell maturation, and on kidney tubular epithelial cells after injury (4, 9, 13, 14, 16, 17). Metalloproteinase-mediated cleavage of TIM-1 at the membrane-proximal region results in the release of a soluble form of TIM-1 which is detectable in the urine and in circulation (18, 19). Urinary TIM-1 is highly elevated in nephropathy and may be a useful biomarker for renal damage (16, 20 - 25).

TIM-1 has been reported to be a receptor for a number of ligands, including phosphatidylserine, leukocyte mono-immunoglobulin-like receptor 5 (LMIR5/CD300b), TIM-1 (homophilic), TIM-4, IgA, and the glycoproteins of a number of enveloped viruses (5, 15, 26-33). Its interaction with phosphatidylserine enables TIM-1 to mediate the phagocytosis of apoptotic cells (26-28). In TIM-1-bearing iNKT cells, interaction with apoptotic cells can also result in iNKT cell activation, proliferation, and cytokine production (11). Interactions between cell-surface or soluble TIM-1 with LMIR5 is proposed to induce LMIR5-mediated activation of myeloid cells including macrophages/monocytes, mast cells, neutrophils, and dendritic cells (29). These interactions contribute to tissue homeostasis and damage during kidney injury (29). Ligand-induced TIM-1 signaling costimulates T cell activation and enhances Th2 cytokine production (9, 31, 34). In humans, TIM-1 serves as a cellular entry receptor for various viruses, including hepatitis A virus, Ebolavirus and Marburgvirus (15, 33).

The Quantikine™ Human Urinary TIM-1/KIM-1/HAVCR Immunoassay is a 4.5 hour solid phase ELISA designed to measure human TIM-1 in urine. It contains NS0-expressed recombinant human TIM-1 and antibodies raised against the recombinant factor. Natural human TIM-1 showed dose-response curves that were parallel to the standard curves obtained using the recombinant Quantikine standards. These results indicate that this kit can be used to determine relative levels of natural human TIM-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human TIM-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TIM-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human TIM-1 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of TIM-1 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- 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.
- If samples generate values higher than the highest standard, dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine™ Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # DKM100	CATALOG # SKM100	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human TIM-1 Microplate	893803	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human TIM-1.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human TIM-1 Conjugate	893804	1 vials	6 vials	21.5 mL of a polyclonal antibody specific for human TIM-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human TIM-1 Standard	893805	1 vial	3 vials	Recombinant human TIM-1 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-82	895375	1 vial	6 vials	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6Q	895128	1 vial	6 vials	21 mL of animal serum with preservatives. *Use diluted 1:2 in this assay.*	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

DKM100 contains sufficient materials to run an ELISA on one 96 well plate.
SKM100 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Squirt bottle, manifold dispenser, or automated microplate washer
- 50 mL and 500 mL graduated cylinders
- Test tubes for dilution of standards
- Human TIM-1 Controls (optional; R&D Systems®, Catalog # QC24)

For the normalization of urine samples, the following is also required:

- Parameter™ Creatinine Assay (R&D Systems, Catalog # KGE005) or equivalent

PRECAUTIONS

Calibrator Diluent RD6Q contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

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

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter. Assay immediately, or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

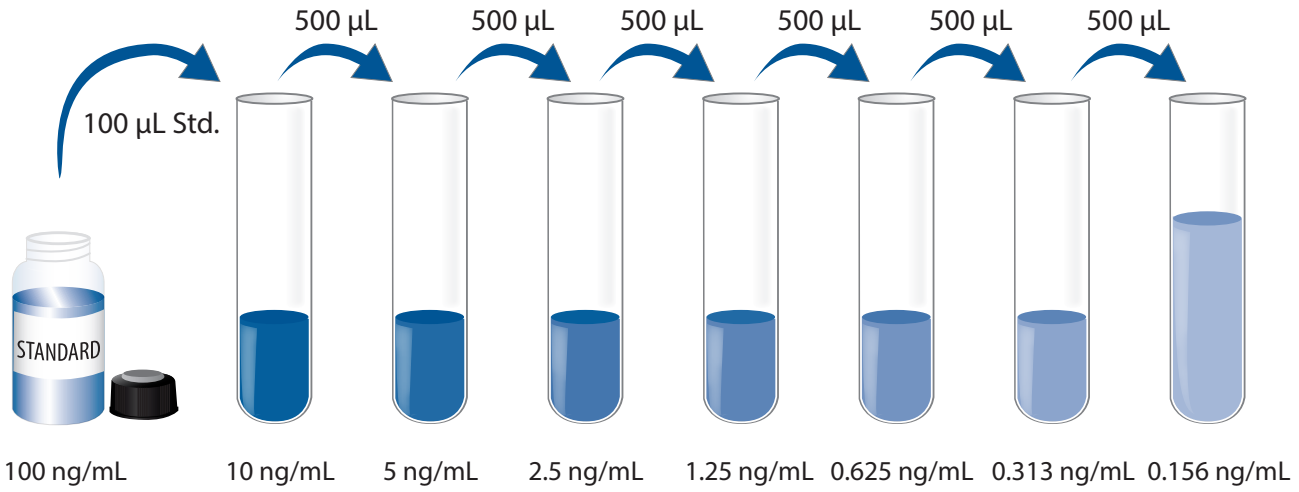
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD6Q (diluted 1:2) - Add 10 mL of Calibrator Diluent RD6Q to 10 mL of deionized or distilled water to prepare 20 mL of Calibrator Diluent RD6Q (diluted 1:2). **Discard after use.**

Human TIM-1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human TIM-1 Standard with deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Mix the standard to ensure complete reconstitution, and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD6Q (diluted 1:2) into the 10 ng/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10 ng/mL standard serves as the high standard. Calibrator Diluent RD6Q (diluted 1:2) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

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

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-82 to each well.
4. Add 50 µL of standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, 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 200 µL of Human TIM-1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 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 the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

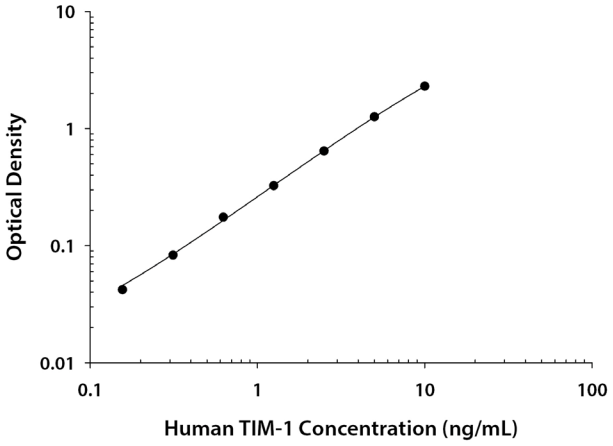
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human TIM-1 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

The amount of human TIM-1 in each sample is normalized to that of the creatinine level. The normalized data is expressed as microgram of TIM-1 per gram of creatinine.

TYPICAL DATA

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



(ng/mL)	O.D.	Average	Corrected
0	0.012 0.012	0.012	—
0.156	0.053 0.055	0.054	0.042
0.313	0.094 0.096	0.095	0.083
0.625	0.187 0.187	0.187	0.175
1.25	0.331 0.343	0.337	0.325
2.5	0.644 0.666	0.655	0.643
5	1.256 1.282	1.269	1.257
10	2.271 2.343	2.307	2.295

PRECISION

Intra-Assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	0.98	3.04	5.88	1.09	3.19	6.23
Standard deviation	0.042	0.119	0.259	0.069	0.193	0.484
CV (%)	4.3	3.9	4.4	6.3	6.1	7.8

RECOVERY

The recovery of human TIM-1 spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Urine (n=20)	104	94-112%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of human TIM-1 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay. For more information on linearity and handling urine specimens, refer to the Evaluation of the Linearity of Quantitative Analytical Methods: A Statistical Approach (NCCLS) publication EP6-A.

		Urine (n=9)
1:2	Average % of Expected	105
	Range (%)	98-112
1:4	Average % of Expected	109
	Range (%)	100-117
1:8	Average % of Expected	110
	Range (%)	101-118
1:16	Average % of Expected	103
	Range (%)	95-110

SENSITIVITY

Thirty-nine assays were evaluated and the minimum detectable dose (MDD) of human TIM-1 ranged from 0.003-0.046 ng/mL. The mean MDD was 0.009 ng/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human TIM-1 produced at R&D Systems®.

SAMPLE VALUES

Urine - Twenty-six samples from apparently healthy volunteers were evaluated for the presence of human TIM-1 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean	Range	Standard Deviation
TIM-1 (ng/mL)	1.35	0.156-5.33	1.09
TIM-1 (µg/g Creatinine)	1.11	0.225-3.20	0.689

SPECIFICITY

This assay recognizes natural and recombinant human TIM-1.

The factors listed below were prepared at 100 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range recombinant human TIM-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

TIM-3
TIM-4

Recombinant mouse:

TIM-1
TIM-4

Recombinant rat:

TIM-1

REFERENCES

1. Freeman, G.J. *et al.* (2010) Immunol. Rev. **235**:172.
2. Waanders, F. *et al.* (2010) J. Pathol. **220**:7.
3. McIntire, J.J. *et al.* (2001) Nat. Immunol. **2**:1109
4. Kuehn, E.W. *et al.* (2002) Am. J. Physiol. Renal Physiol. **283**:F1326.
5. Kim, H.Y. *et al.* (2011) J. Clin. Invest. **121**:1111.
6. Ding, Q. *et al.* (2011) J. Clin. Invest. **121**:3645.
7. Ma, J. *et al.* (2011) Biochem. Biophys. Res. Commun. **406**:223.
8. de Souza, A.J. *et al.* (2005) Proc. Natl. Acad. Sci. USA **102**:17113.
9. Umetsu, S.E. *et al.* (2005) Nat. Immunol. **6**:447.
10. Nakae, S. *et al.* (2007) Blood **110**:2565.
11. Lee, H.H. *et al.* (2010) J. Immunol. **185**:5225.
12. Kim, H.S. *et al.* (2010) J. Immunol. **184**:4095.
13. Xiao, S. *et al.* (2011) Eur. J. Immunol. **41**:1539.
14. Ichimura, T. *et al.* (1998) J. Biol. Chem. **273**:4135.
15. Kondratowicz, A.S. *et al.* (2011) Proc. Natl. Acad. Sci. USA **108**:8426.
16. Ichimura, T. *et al.* (2003) Am. J. Physiol. Renal Physiol. **286**:F552.
17. van Timmeren, M.M. *et al.* (2006) Am. J. Physiol. Renal Physiol. **291**:F456.
18. Zhang, Z. *et al.* (2007) J. Am. Soc. Nephrol. **18**:2704.
19. Bailly, V. *et al.* (2002) J. Biol. Chem. **277**:39739.
20. Vaidya, V.S. *et al.* (2006) Am. J. Physiol. Renal Physiol. **290**:F517.
21. Kramer, A.B. *et al.* (2009) Am. J. Physiol. Renal Physiol. **296**:F1136.
22. Prozialeck, W.C. *et al.* (2009) Toxicol. Appl. Pharmacol. **238**:306.
23. Han, W.K. *et al.* (2002) Kidney Int. **62**:237.
24. Wasilewska, A. *et al.* (2011) Pediatr. Nephrol. **26**:579.
25. Nielsen, S.E. *et al.* (2010) Diabet. Med. **27**:1144.
26. Miyanishi, M. *et al.* (2007) Nature **450**:435.
27. Kobayashi, N. *et al.* (2007) Immunity **27**:927.
28. Ichimura, T. *et al.* (2008) J. Clin. Invest. **118**:1657.
29. Yamanishi, Y. *et al.* (2010) J. Exp. Med. **207**:1501.
30. Santiago, C. *et al.* (2007) Immunity **26**:299.
31. Meyers, J.H. *et al.* (2005) Nat. Immunol. **6**:455.
32. Tami, C. *et al.* (2007) J. Virol. **81**:3437.
33. Feigelstock, D. *et al.* (1998) J. Virol. **72**:6621.
34. Binne, L.L. *et al.* (2007) J. Immunol. **178**:4342.