MSD® MULTI-ARRAY Assay System

Rat TIM-1/KIM-1/HAVCR Kit

1-Plate Kit	K153JHC-1
5-Plate Kit	K153JHC-2
25-Plate Kit	K153JHC-4

MSD Toxicology Assays

Rat TIM-1/KIM-1/HAVCR Kit

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

MESO SCALE DISCOVERY®

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Ordering Information

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Introduction

T cell immunoglobulin and mucin domain containing molecule 1(TIM-1/KIM-1/HAVCR) is a type 1 transmembrane glycoprotein found on $CD4^+$ T cells and renal proximal tubule epithelial cells. The extracellular domain of TIM-1 is made of an immunoglobulin-like domain topping a long mucin-like domain, suggesting a possible role in cell adhesion. TIM-1 is released upon certain types of acute kidney injury and can be measured in urine, serum, or plasma.

Principle of the Assay

MSD toxicology assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. The Rat TIM-1/KIM-1/HAVCR assay has been qualified according to the principles outlined in "Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement" by Lee, J.W. et al.[1] The Rat TIM-1 kit is a sandwich immunoassay (Figure 1). MSD provides a plate pre-coated with capture antibodies. The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) over the course of one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface; recruitment of the detection antibodies by the bound analytes completes the sandwich. The user adds an MSD buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD instrument where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light to provide a quantitative measure of analytes in the sample.

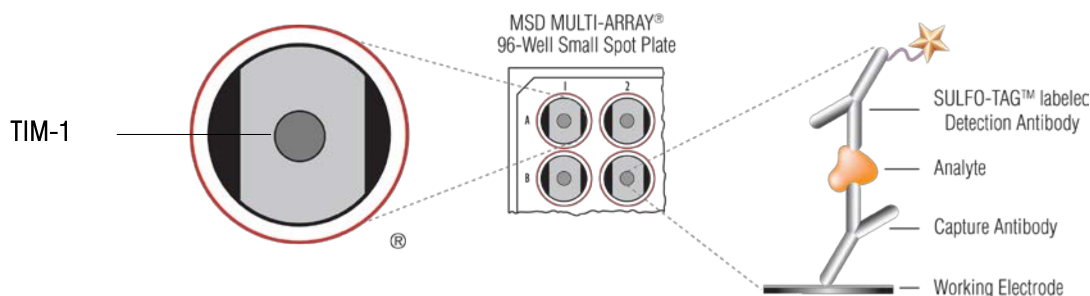


Figure 1. Spot diagram showing placement of analyte capture antibody. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

Reagents Supplied

Product Description	Storage	Quantity per Kit K153JHC-1	K153JHC-2	K153JHC-4
MULTI-ARRAY 96-Well Small Spot Rat TIM-1/KIM-1/HAVCR Plate L453MGA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-rat TIM-1//KIM-1/HAVCR Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Rat TIM-1//KIM-1/HAVCR Calibrator (20X)	≤-70°C	1 vial (15 µL)	5 vials (15 µL ea)	25 vials (15 µL ea)
Diluent 5 R52BA-5 (25 mL)	≤-10°C	1 bottle (25 mL)	3 bottles (25 mL ea)	15 bottles (25 mL ea)
Blocker A Kit [Blocker A (dry) in 250 mL bottle and 50 mL bottle of 5X Phosphate Buffer] R93AA-2 (250 mL)	RT	1 kit (250 mL)	1 kit (250 mL)	5 kits (250 mL ea)
Read Buffer T (4X) R92TC-3 (50 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Microcentrifuge tubes for preparing serial dilutions
- ❑ Phosphate buffered saline plus 0.05% Tween-20 (PBS-T) for plate washing
- ❑ Liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL/well into a 96-well microtiter plate
- ❑ Plate washing equipment: automated plate washer or multichannel pipette
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker
- ❑ Deionized water

Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines. Additional product-specific safety information is available in the safety data sheet (SDS), which can be obtained from MSD Customer Service.

[1] SULFO-TAG–conjugated detection antibodies should be stored in the dark.

Reagent Preparation

Bring all reagents to room temperature. Thaw the stock calibrator on ice.

> **Important:** Upon first thaw, separate Diluent 5 into aliquots appropriate for the size of your needs before refreezing. This diluent can go through up to three freeze-thaw cycles without significantly affecting the performance of the assay.

Prepare Blocker A Solution

Follow the Blocker A instructions included in the kit.

Prepare Calibrator Dilutions

MSD supplies a calibrator for the Rat TIM-1 assay at 20-fold higher concentration than the recommended highest standard. We recommend a 7-point standard curve with 3-fold serial dilution steps and a zero calibrator blank. Thaw the stock calibrator and keep on ice, then add to diluent at room temperature to make the standard curve solutions. To view the actual concentration of the calibrator, refer to the certificate of analysis (COA) supplied with the kit. You may also find a copy of the lot-specific COA at www.mesoscale.com by entering K153JHC in the search box.

To prepare 7 standard solutions plus a zero calibrator blank for up to 4 replicates:

1) Prepare the highest standard by adding 10 µL of the stock calibrator to 190 µL of Diluent 5. Mix well.
2) Prepare the next standard by transferring 80 µL of the highest standard to 160 µL of Diluent 5. Mix well. Repeat 3-fold serial dilutions 5 additional times to generate 7 standards.
3) Use Diluent 5 as the zero calibrator.

Dilute Samples

Rat samples may need to be diluted prior to the assay in order to get the analyte levels into the detection range. A 5-fold dilution of urine samples into Diluent 5 is recommended for this assay. Depending on the sample set under investigation, higher or lower dilution factors may be necessary.

Prepare Detection Antibody Solution

MSD provides the detection antibody as a 50X stock solution. The working detection antibody solution is 1X.

For 1 plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-rat TIM-1/KIM-1/HAVCR Antibody
- ❑ 2940 µL of Diluent 5

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 1X.

For 1 plate, combine:

- ❑ 5 mL of Read Buffer T (4X)
- ❑ 15 mL of deionized water

You may prepare diluted read buffer in advance and store it at room temperature in a tightly sealed container.

Prepare MSD Plate

MSD plates are pre-coated with capture antibodies (Figure 1) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. Plates can be used as delivered; no additional preparation (e.g., pre-wetting) is required.

Protocol

1. **Add Blocker A Solution:** Add 150 µL of Blocker A solution to each well. Seal the plate with an adhesive plate seal and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.

2. **Wash and Add Sample:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 25 µL of Diluent 5 per well. Then, add 25 µL of sample (standards or unknowns) per well. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.

   You may prepare detection antibody solution during incubation.

3. **Wash and Add Detection Antibody Solution:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 25 µL of 1X detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.

   You may prepare diluted read buffer during incubation.

4. **Wash and Read:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 150 µL of 1X Read Buffer T to each well. Analyze the plate on an MSD instrument. No incubation in read buffer is required before reading the plate.

Notes

Shaking the plate typically accelerates capture at the working electrode.

You may keep excess diluted read buffer in a tightly sealed container at room temperature for later use.

Bubbles introduced when adding read buffer will interfere with imaging of the plate and produce unreliable data. Use reverse pipetting technique to avoid creating bubbles.

Due to the varying nature of each research application, you should assess assay stability before allowing plates to sit with read buffer for extended periods.

Analysis of Results

The calibrators should be run in duplicate to generate a standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from samples with known levels of the analyte of interest can be used to calculate the concentration of analyte in the sample. The assays have a wide dynamic range (3–4 logs) which allows accurate quantification in many samples without the need for dilution. The MSD DISCOVERY WORKBENCH® analysis software utilizes a 4-parameter logistic model (or sigmoidal dose-response) and includes a 1/Y2 weighting function. The weighting function is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

Assay Validation and Verification

The performance of this kit meets levels of consistency and robustness as determined by methods based on the principles outlined in "Fit -for-Purpose Method Development and Validation for Successful Biomarker Measurement" by Lee, J.W. et al.[1]

Bioanalytical and functional characterizations of calibrators, antibodies and assay components are completed to allow for bridging of reagents between lots. This includes plate coating uniformity and reagent and component specificity testing for individual kit lots.

Control samples for specific matrices are designed and tested to meet the accuracy, precision and sensitivity criteria for a kit that has completed the validation process. Spike recovery and dilution linearity of endogenous samples, pooled and individual matrices, are tested across the assay range.

- Sensitivity, Range, and Curve Fitting
    - Sample range and assay sensitivity are established from 4-parameter logistic fitted calibration curves with $1/Y^2$ weighting. Percent recovery of calibrators and controls between the upper limit of quantification (ULOQ) and lower limit of quantification (LLOQ) must have calculated concentration %CV of less than 20% and accuracy within 20% of the expected concentration.
    - The limits of quantification defined in the product insert are verified for each lot as part of the lot verification and quality control release.

- Accuracy and Precision

High, mid, and low controls made in matrix (defined on a kit-by-kit basis) are run to measure accuracy and precision.

- Validation – The assay is tested over multiple days (>6 days) and multiple runs per day for a total of 15-20 runs of complete kits. Precision is measured for the standard curve for intra- and inter-day coefficients of variance (CVs) of less than 20%. The typical specification includes a calculated concentration CV of less than 20%, accuracy within 20% of expected concentration, and a total error of less than 30%. The kit specifications for this lot are provided in the enclosed COA.
- Verification – A multi-day (2-3 days) analysis with multiple runs per day of 6-12 total plates is performed as part of the release testing for each lot. The specifications for release are provided in the COA.

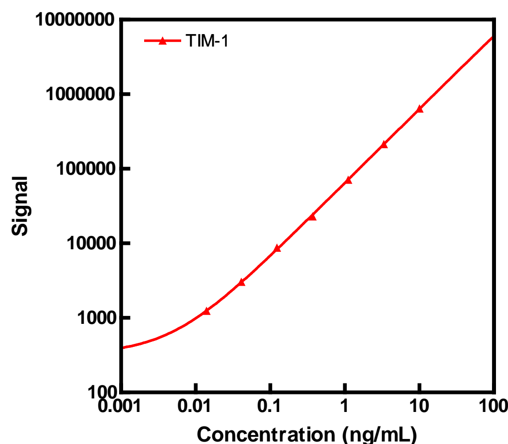
- Robustness and Stability

Freeze-thaw testing and accelerated stability studies performed during assay development (calibrators, antibodies, controls) are augmented with real-time stability studies on complete kits out to 18 months from the date of manufacture.

All acceptance criteria and verification conformance are defined in the COA for all kit lots. Presented below are representative data from the assay validation for this assay that meets the criteria described above. The kit lot-specific standard curve and measured limits of quantification can be found in the COA enclosed with the kit.

Typical Data

The following standard curve graph illustrates the dynamic range of the assay. Actual signals will vary. Best quantification of unknown samples will be achieved by generating a standard curve for each plate using a minimum of 2 replicates of standards. For each kit lot, refer to the COA for the actual concentration of the calibrator.



	TIM-1	
Conc. (ng/mL)	Average Signal	%CV
0	259	5.1
0.0137	1237	2.7
0.0412	3036	3.1
0.123	8727	3.3
0.370	22 906	5.1
1.11	70 952	6.0
3.33	212 959	2.6
10	641 896	3.3

Sensitivity

The lower limit of detection (LLOD) is the calculated concentration of the signal that is 2.5 standard deviations over the zero calibrator.

A multi-plate, multi-day study was performed to measure the reproducibility of the assay. The lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) were established from the multiple plate run.

The LLOQ is determined as the lowest concentration where the %CV of the calculated concentration is less than 20% and the percent recovery of the standard is between 80% and 120%.

The ULOQ is determined as the highest concentration where the %CV of the calculated concentration is less than 20% and the percent recovery of the standard is between 80% and 120%.

	TIM-1 (ng/mL)
LLOD	0.001
LLOQ	0.02
ULOQ	10

Precision

High, mid, and low controls were made by spiking recombinant protein into pooled rat urine. The low control was the endogenous pooled rat urine. The controls were run at a 10-fold dilution.

The controls were run in quadruplicate on each of 9 plates run across multiple days (n>3).

Average Intra-plate % CV is the average percent CV of the control replicates within an individual plate.
Inter-plate %CV is the variability of controls across 9 plates over 3 days.

Inter-lot %CV is the variability of controls across 5 kit lots.

	Control	Runs	Average Conc. (ng/mL)	Average Intra-plate %CV	Inter-plate %CV	Inter-lot %CV
TIM-1	High	9	0.967	3.8	5.6	12.2
	Mid	9	0.315	3.7	4.8	8.9
	Low	9	0.132	3.8	4.3	8.7

Dilution Linearity

To assess linearity, rat urine samples were diluted 5-fold, 10-fold, 20-fold, 40-fold, and 80-fold prior to testing. The concentrations shown below have been corrected for dilution (concentration = measured x dilution factor). Percent recovery is calculated as the measured concentration divided by the concentration measured from the previous dilution (expected).

% Recovery= (measured∗dilution factor)/expected∗100

		TIM-1		
Sample Type	Fold Dilution	Conc. (ng/mL)	Conc. %CV	% Recovery
Urine	1	0.495	9.7	
	5	0.741	2.5	149
	10	0.739	17.9	100
	20	0.858	17.6	116
	40	0.814	7.9	95
	80	0.766	20.7	94

Spike Recovery

Rat urine samples were spiked with the calibrator at multiple values throughout the range of the assay. The samples were diluted 5-fold and then spiked with calibrator at the levels indicated in the table below.

% Recovery=measured/expected∗100

	TIM-1			
Sample Type	Spike Conc. (ng/mL)	Measured Conc. (ng/mL)	Measured Conc. %CV	% Recovery
Urine	0	0.0477	3.7	
	0.156	0.230	1.3	112
	0.312	0.387	0.7	107
	0.625	0.668	5.0	99
	2.50	2.37	5.9	93
	10	8.98	7.2	89

Samples

Urine samples were collected from normal Sprague-Dawley rats, diluted 10-fold, and tested with the Rat TIM-1 assay. Median and range of concentrations are displayed below. Concentrations are corrected for sample dilution.

Sample Type	Statistic	TIM-1
Urine	Median (ng/mL)	1.50
	Range (ng/mL)	0.708–4.37
	Number of Samples	6

Assay Components

Calibrator

Recombinant rat TIM-1/KIM-1/HAVCR (residues 18-238) was expressed in murine myeloma cells.
This analyte was calibrated against an internal control and diluted to make the final Rat TIM-1/KIM-1/HAVCR Calibrator.

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
TIM-1	Goat Polyclonal	Goat Polyclonal

References

1. Lee JW, Devanarayan V, Barrett YC, Weiner R, Allinson J, Fountain S, Keller S, Weinryb I, Green M, Duan L, Rogers JA, Millham R, O'Brien PJ, Sailstad J, Khan M, Ray C, Wagner JA. Fit-for-purpose method development and validation for successful biomarker measurement. Pharm Res. 2006 Feb;23(2):312-28.
2. Dieterle F, Marrer E, Suzuki E, Grenet O, Cordier A, Vonderscher J. Monitoring kidney safety in drug development: emerging technologies and their implications. Curr. Opin. Drug Discov. Devel.2008;11(1):60-71

Summary Protocol

MSD 96-well MULTI-ARRAY Rat TIM-1 Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the Rat TIM-1 assay.

Sample and Reagent Preparation

- ❑ Bring all reagents to room temperature and thaw the calibrator on ice.
- ❑ Prepare Blocker A solution.
- ❑ Prepare an 8-point standard curve using the supplied calibrator:
  - Dilute the stock calibrator 20-fold in Diluent 5.
  - Perform a series of 3-fold dilution steps and prepare a zero calibrator blank.
- ❑ If necessary, dilute samples 5-fold in Diluent 5 before adding to the plate.
- ❑ Prepare detection antibody solution by diluting stock detection antibody 50-fold in Diluent 5.
- ❑ Prepare 1X Read Buffer T by diluting stock 4X Read Buffer T 4-fold with deionized water.

Step 1 : Add Blocker A Solution

- ❑ Add 150 µL/well of Blocker A Solution.
- ❑ Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 2 : Wash and Add Sample

- ❑ Wash plate 3 times with 300 µL/well of PBS-T.
- ❑ Add 25 µL/well of Diluent 5.
- ❑ Add 25 µL/well of sample (standards or unknowns).
- ❑ Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 3 : Wash and Add Detection Antibody Solution

- ❑ Wash plate 3 times with 300 µL/well of PBS-T.
- ❑ Add 25 µL/well of 1X detection antibody solution.
- ❑ Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 4 : Wash and Read Plate

- ❑ Wash plate 3 times with 300 µL/well of PBS-T.
- ❑ Add 150 µL/well of 1X Read Buffer T.
- ❑ Analyze plate on an MSD instrument.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

	1	2	3	4	5	6	7	8	9	10	11	12
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