

Meso Scale Discovery

MULTI-ARRAY[®] Assay System

Rat TIM-1/KIM-1/HAVCR Assay Kit

1-Plate Kit

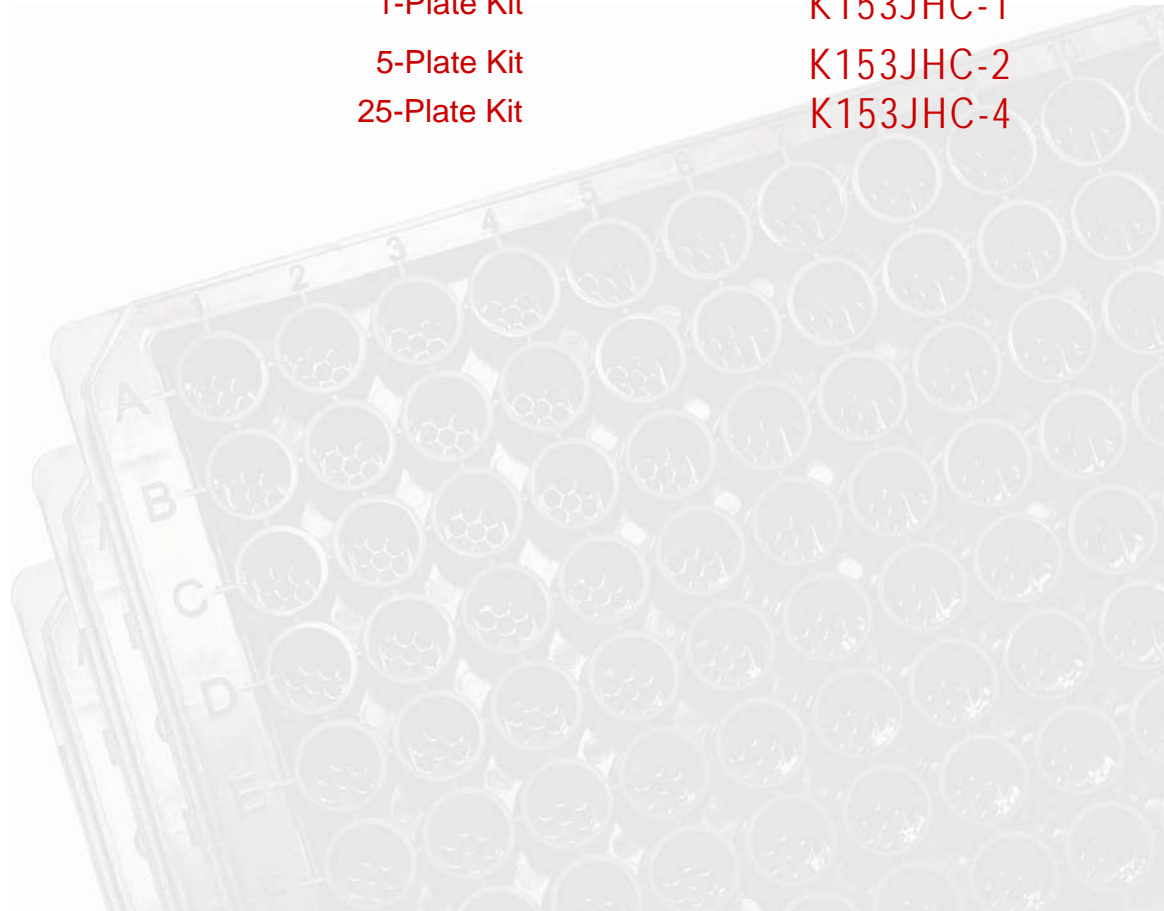
K153JHC-1

5-Plate Kit

K153JHC-2

25-Plate Kit

K153JHC-4



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MSD Toxicology Assays

Rat TIM-1/KIM-1/HAVCR Assay Kit

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.

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Ordering Information

ordering information

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Introduction

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

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 assays are available in both singleplex and multiplex formats. In a singleplex assay, an antibody for a specific protein target is coated on one electrode (or “spot”) per well. In a multiplex assay, an array of capture antibodies against different targets is patterned on distinct spots in the same well. Our Rat TIM-1/KIM-1/HAVCR Assay detects TIM-1 in a sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with TIM-1 antibody. The user adds the sample and a solution containing the labeled detection antibody—anti-rat TIM-1 labeled with an electrochemiluminescent compound, MSD SULFO-TAG[™] label—over the course of one or more incubation periods. TIM-1 in the sample binds to capture antibody immobilized on the working electrode surface; recruitment of the labeled detection antibody by bound analyte completes the sandwich. The user adds an MSD read buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD SECTOR[®] instrument for analysis. Inside the SECTOR instrument, a voltage applied to the plate electrodes causes the labels bound to the electrode surface to emit light. The instrument measures intensity of emitted light to afford a quantitative measure of TIM-1 present in the sample.

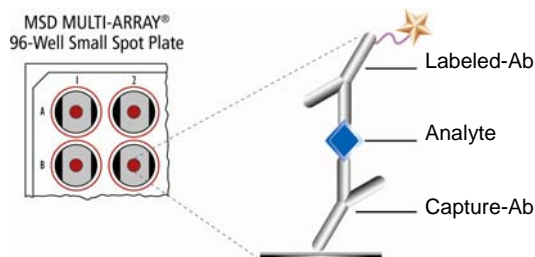


Figure 1. Sandwich immunoassay on MSD platform. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.



Reagents Supplied

reagents supplied

Product Description	Storage	Quantity per Kit		
		K153JHC-1	K153JHC-2	K153JHC-4
MULTI-ARRAY 96-well Rat TIM-1/KIM-1/HAVCR Plate(s) L453JHA-1	2-8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-rat TIM-1/KIM-1/HAVCR Antibody (50X) ¹	2-8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Rat TIM-1/KIM-1/HAVCR Calibrator (20X) 200 ng/mL	≤ -70°C	1 vial (15 µL)	5 vials (15 µL ea)	25 vials (15 µL ea)
Blocker A kit R93AA-2 (250 mL)	RT	1 bottle (250 mL)	1 bottle (250 mL)	5 bottles (250 mL ea)
Mouse/Rat Serum Cytokine (MRSC) Antibody Diluent R52BA-5 (25 mL)	-20°C	1 bottle (25 mL)	3 bottle (25 mL)	15 bottles (25 mL ea)
Read Buffer T (with surfactant), 4X R92TC-3 (50 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)



Required Materials and Equipment — not supplied

required materials and equipment — not supplied

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



Safety

safety

Safe laboratory practices and personal protective equipment such as gloves, safety glasses, and lab coats should be used at all times during the handling of all kit components. All hazardous samples should be handled and disposed of properly, in accordance with local, state, and federal guidelines.

¹ Some SULFO-TAG labeled detection antibodies may be light-sensitive, so they should be stored in the dark.

VI

Reagent Preparation

reagent preparation

Bring all reagents to room temperature. The stock calibrator should be thawed on ice.

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

Prepare Blocker A Solution

Follow instructions included with the Blocker A Kit.

Prepare Calibrator and Control Solutions

Calibrator for the Rat TIM-1 assay is supplied at 20-fold higher concentration than the recommended highest calibrator. For the assay, an 8-point standard curve is recommended with 3-fold serial dilution steps and a zero calibrator. The table below shows the concentrations of the 8-point standard curve:

Standard	TIM-1 conc. (ng/mL)	Dilution Factor
Stock Cal. Vial	200	
STD-01	10	20
STD-02	3.33	3
STD-03	1.11	3
STD-04	0.370	3
STD-05	0.123	3
STD-06	0.041	3
STD-07	0.014	3
STD-08	0	n/a

To prepare this 8-point standard curve:

- 1) Prepare the highest Calibrator by adding 10 μ L of the Calibrator stock vial to 190 μ L of MRSC Antibody Diluent (20-fold dilution).
- 2) Prepare the next Calibrator by transferring 75 μ L of the diluted Calibrator to 150 μ L of MRSC Antibody Diluent. Repeat 3-fold serial dilutions 5 additional times to generate 7 Calibrators.
- 3) Reserve 150 μ L of MRSC Antibody Diluent to be used as zero calibrator.

Calibrators should be prepared at room temperature no more than 20 minutes before addition to the assay plate.

Dilution of Samples

Some rat samples may need to be diluted prior to the assay in order to get the analyte levels into the detection range. If this is the case, Mouse/Rat Serum Cytokine Antibody Diluent should be used to dilute samples. A 5-fold dilution of urine samples is recommended. Depending on the sample set under investigation, higher or lower dilution factors may be necessary.

Prepare Detection Antibody Solution

The Detection Antibody is provided as a 50X stock of Anti-rat TIM-1 Antibody. The working Detection Antibody Solution should contain 1X as final concentration. For each plate used, dilute 60 μ L of the stock Detection Antibody stock into a final volume of 3 mL of MRSC Antibody Diluent.

Prepare Read Buffer

The Read Buffer should be diluted in deionized water to make a final concentration of 1X Read Buffer T. Add 5 mL of stock Read Buffer T (4X) to 15 mL of deionized water for each plate.

Prepare MSD Plate

This plate has been pre-coated with antibody as shown in Figure 1. The plate can be used as delivered; no additional preparation (e.g., pre-wetting) is required. The plate has also been exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies.

VII Assay Protocol

assay protocol

1. **Addition of Blocker A Solution:** Dispense 150 μL of Blocker A Solution into 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 Addition of Sample or Calibrator:** Wash the plate 3X with PBS-T. First, dispense 25 μL of MRSC Antibody Diluent into each well of the MSD plate. Then, dispense 25 μL of diluted sample or Calibrator into the appropriate wells of the MSD plate. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
3. **Wash and Addition of the Detection Antibody Solution:** Wash the plate 3X with PBS-T. Dispense 25 μL of the 1X Detection Antibody Solution into each well of the MSD plate. Seal the plate and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
4. **Wash and Read:** Wash the plate 3X with PBS-T. Add 150 μL of 1X Read Buffer T to each well of the MSD plate. Analyze the plate on the SECTOR Imager. Plates may be read immediately after the addition of Read Buffer.

Notes

Shaking a 96-well MSD MULTI-ARRAY plate typically accelerates capture at the working electrode.

Bubbles in the fluid will interfere with reliable reading of MULTI-ARRAY plate. Use reverse pipetting techniques to insure bubbles are not created when dispensing the Read Buffer.

VIII Analysis of Results

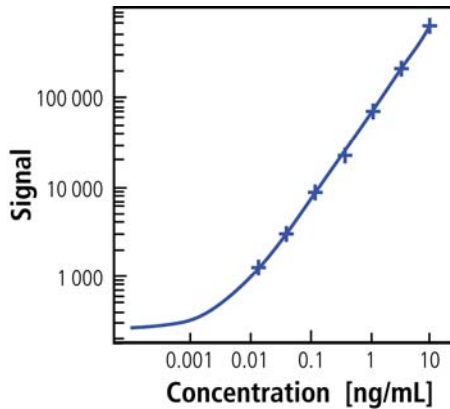
analysis of results

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 quantitation 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 $1/Y^2$ weighting function. The weighting functionality is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

IX Typical Standard Curve

typical standard curve

The following standard curve is an example of the dynamic range of the assay. The actual signals may vary and a standard curve should be run for each set of samples and on each plate for the best quantitation of unknown samples.



Conc. (ng/mL)	Mean	%CV
0	259	5.1
0.014	1237	2.7
0.041	3036	3.1
0.123	8727	3.3
0.370	22906	5.1
1.11	70952	6.0
3.33	212959	2.6
10	641896	3.3

X Sensitivity

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 quantitation (LLOQ) and upper limit of quantitation (ULOQ) were established from the multiple plate run.

The LLOQ is determined as the lowest standard 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 standard 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

XI Precision

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 in triplicate on each of 9 plates run across three days. The controls were run at a 10-fold dilution.

	Control	Plates	Avg. Conc. (ng/mL)	Intra-Plate			Inter-Plate
				Average %CV	Max %CV	Min %CV	%CV
TIM-1	High	9	0.821	6.8	15.8	1.3	14.0
	Mid	9	0.298	3.8	7.7	1.5	13.6
	Low	9	0.118	4.3	8.5	2.2	14.5

XII Spike Recovery

spike recovery

Rat urine samples were spiked with the standards at multiple levels throughout the range of the assay. The spiked samples were tested at a 5-fold dilution into the assay diluent.

% Recovery = measured/expected x 100

TIM-1 Spike Level (ng/mL)	Expected Conc. (ng/mL)	Measured Conc. (ng/mL)	%CV	% Recovery
10	10.05	8.98	7.2	89
2.5	2.55	2.37	5.9	93
0.625	0.675	0.67	5.0	99
0.312	0.362	0.39	0.7	107
0.156	0.156	0.23	1.3	112
0	0.05	0.05	3.7	

XIII Linearity

linearity

Serial dilutions of a rat urine sample were tested to assess linearity. At 80-fold dilution, the concentration was below the assay LLOQ.

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

TIM-1				
Dilution	Signal	Adjusted Conc. (ng/mL)	%CV	% Recovery
1	9190	0.495	9.7	
5	2752	0.741	2.5	149
10	1443	0.739	17.9	100
20	913	0.858	17.6	116
40	540	0.814	7.9	95
80	368	0.766	20.7	94

XIV Calibrators

calibrators

Recombinant rat TIM-1/KIM-1/HAVCR (residues 18–238) was expressed in murine myeloma cell line, NSO. This analyte was calibrated against an internal control and diluted to a final concentration of 200 ng/mL to make the Rat TIM-1/KIM-1/HAVCR Calibrator.

XV Reference

reference

Dieterle F, Marrer E, Suzuki E, Grenet O, Cordier A, Vonderscher J. (2008) *Monitoring kidney safety in drug development: emerging technologies and their implications*. *Curr. Opin. Drug Discov. Devel.* 11(1): 60-71

Summary Protocol

MSD 96-well MULTI-ARRAY Rat TIM-1/KIM-1/HAVCR Assay Kit

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

Step 1 : Sample and Reagent Preparation

Bring appropriate diluents and plates to room temperature

If necessary, samples should be diluted in MRSC Antibody Diluent.

Prepare Blocker A Solution.

Prepare an 8-point standard curve using supplied calibrator:

- The Calibrator should be diluted in MRSC Antibody Diluent.
- Dilute the stock Calibrator 1:20 in MRSC Antibody Diluent, then perform a series of 3-fold dilution steps and a no calibrator blank.

Prepare Detection Antibody Solution by diluting the 50X Anti-rat TIM-1 Antibody to 1X in 3.0 mL of MRSC Antibody Diluent per plate.

Prepare 20 mL of 1X Read Buffer T by diluting MSD Read Buffer T (with surfactant), 4X with deionized water.

Step 2 : Add Blocker A Solution

Dispense 150 μ L/well Blocker A Solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 3 : Wash and Add Sample or Calibrator

Wash plate 3X with PBS-T.

Dispense 25 μ L/well MRSC Antibody Diluent.

Dispense 25 μ L/well Calibrator or Sample.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 4 : Wash and Add Detection Antibody Solution

Wash plate 3X with PBS-T.

Dispense 25 μ L/well 1X Detection Antibody Solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 5 : Wash and Read Plate

Wash plate 3X with PBS-T.

Dispense 150 μ L/well 1X Read Buffer T.

Analyze plate on SECTOR instrument.

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