

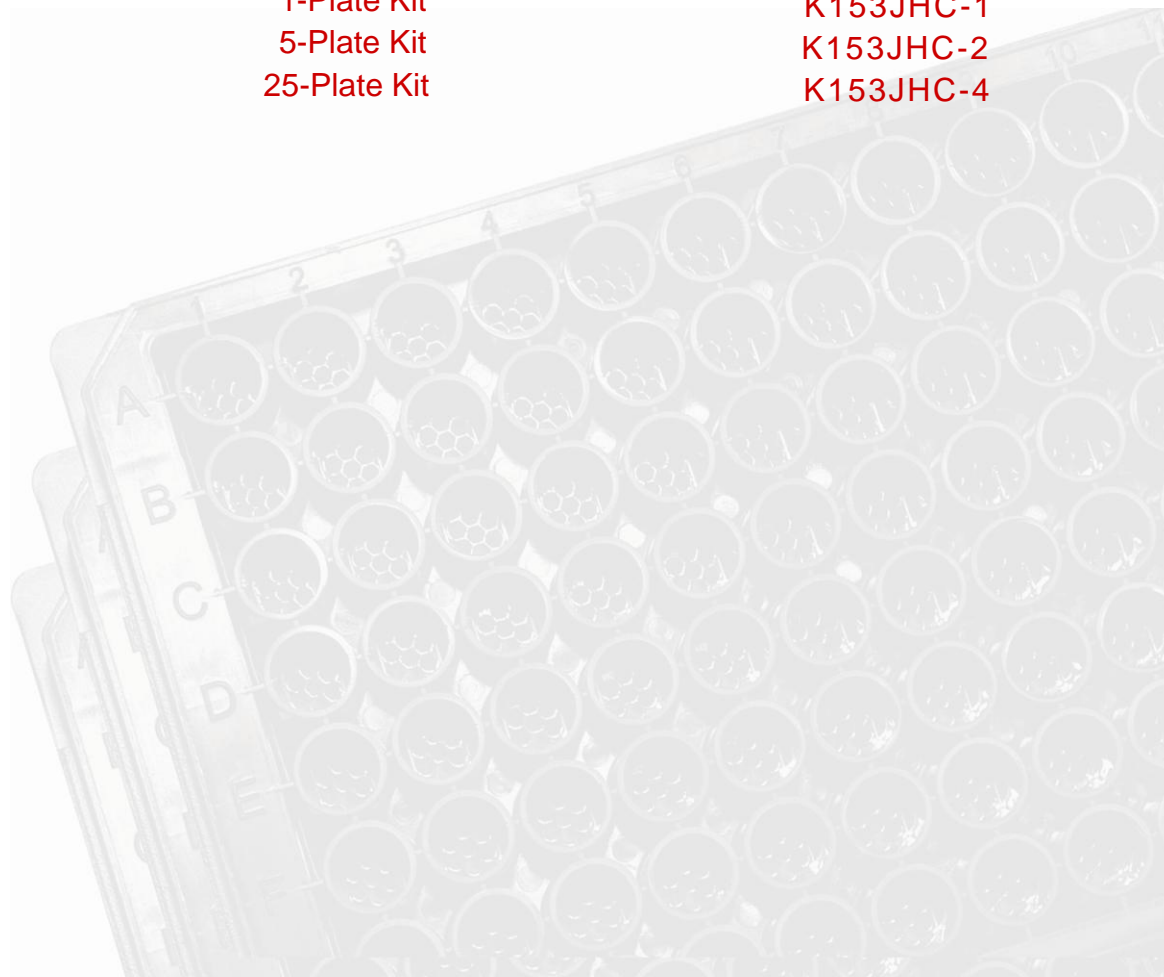
MESO SCALE DISCOVERY

MULTI-ARRAY Assay System

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

1-Plate Kit
5-Plate Kit
25-Plate Kit

K153JHC-1
K153JHC-2
K153JHC-4



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MSD Toxicology Assays

Rat TIM-1/KIM-1/HAVCR Assay

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

ordering information

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MSD Advantage

M S D a d v a n t a g e

MESO SCALE DISCOVERY'S MULTI-ARRAY[®] Technology is a multiplex immunoassay system that enables the measurement of biomarkers utilizing the next generation of electrochemiluminescent detection. In an MSD[®] assay, specific Capture Antibodies for the analytes are coated in arrays in each well of a 96-well carbon electrode plate surface. The detection system uses patented SULFO-TAG[™] labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of the MULTI-ARRAY and MULTI-SPOT[®] plates. The electrical stimulation is decoupled from the output signal, which is light, to generate assays with minimal background. MSD labels can be conveniently conjugated to biological molecules, are stable and are non-radioactive. Additionally, only labels near the electrode surface are detected, enabling non-washed assays.

One of the advantages of MSD assays is the minimal sample volume required as compared to a traditional ELISA, which is also limited by its inability to measure more than a single analyte. With an MSD assay, ten different biomarkers can be analyzed simultaneously using as little as 10-25 μ L of sample. These assays have high sensitivity, up to five logs of linear dynamic range, and excellent performance in complex biological matrices. Combined, these advantages enable the measurement of native levels of biomarkers in normal and diseased samples without multiple dilutions. Further, the simple and rapid protocols of MSD assays provide a powerful tool to generate reproducible and reliable results. The MSD product line offers a diverse menu of assay kits for profiling biomarkers, cell signaling pathways, and other applications, as well as a variety of plates and reagents for assay development.

Introduction

i n t r o d u c t i o n

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. These assays have been qualified according to the principles outlined in “Fit-for-Purpose Method Development and Validation for Successful Biomarker Measurement” by Lee, J.W. et al¹. 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 is a sandwich immunoassay (Figure 1). MSD provides a plate that has been pre-coated with capture antibody for TIM-1. The user adds the sample and a solution containing the conjugated detection antibody—anti-TIM-1 conjugated with an electrochemiluminescent compound, MSD SULFO-TAG™ label—over the course of one or more incubation periods. Analyte in the sample binds to the capture antibody immobilized on the working electrode surface; recruitment of the conjugated detection antibody by bound analytes 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 provide a quantitative measure of TIM-1, present in the sample.

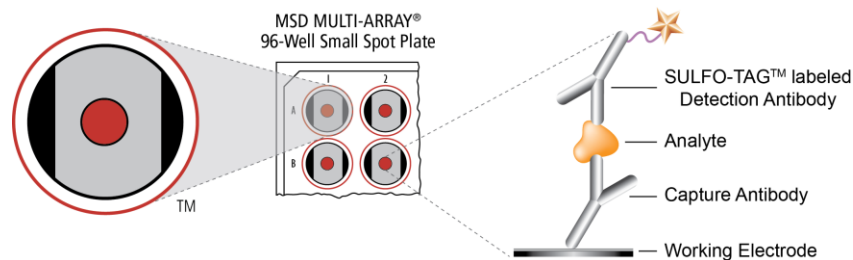


Figure 1. Spot diagram showing placement of analyte capture antibody. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

IV 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)	≤ -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)
Diluent 5 R52BA-5 (25 mL)	≤ -10°C	1 bottle (25 mL)	3 bottle (25 mL)	15 bottles (25 mL ea)
Read Buffer T (4X) R92TC-3 (50 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)

V 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

VI 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.

VII Reagent Preparation

reagent preparation

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

Important: Upon first thaw, separate Diluent 5 into aliquots appropriate to the size of your assay needs. These diluents 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 is supplied at 20-fold higher concentration than the recommended highest Calibrator. An 8-point standard curve is recommended with 3-fold serial dilution steps and a zero Calibrator. The stock Calibrator should be thawed and kept on ice, but should be added into diluent at room temperature to make the standard curve solutions. For the actual concentration of the Calibrator, refer to the certificate of analysis (C of A) supplied with the kit. A copy of the kit specific C of A can also be found at www.mesoscale.com

To prepare this 8-point standard curve for up to 3 replicates:

- 1) Prepare the highest Calibrator by adding 10 μL of the Calibrator stock vial to 190 μL of Diluent 5.
- 2) Prepare the next Calibrator by transferring 80 μL of the diluted Calibrator to 160 μL of Diluent 5. Repeat 3-fold serial dilutions 5 additional times to generate 7 Calibrators.
- 3) The recommended 8th Standard is Diluent 5 (i.e. zero Calibrator).

Calibrators should be prepared at room temperature no more than 20 minutes before use.

Dilution of 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

The Detection Antibody is provided as a 50X stock solution. The final concentration of the working Detection Antibody Solution should be at 1X. For each plate used, dilute a 60 μL aliquot of the stock Detection Antibody into 2940 μL of Diluent 5.

Prepare Read Buffer

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

Prepare MSD Plate

This plate has been pre-coated with antibody for the analyte 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.

VIII 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 the Sample or Calibrator:** Wash the plate 3 times with PBS-T. Dispense 25 μL of Diluent 5 into each well of the MSD plate. Then, dispense 25 μL of diluted sample or Calibrator into separate 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 3 times 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 3 times 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. No incubation in Read Buffer is required before reading the plate.

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.

IX 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 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 $1/Y^2$ 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.

X Assay Qualification and Verification

assay qualification 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 qualification 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-PL 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 (need to be defined on a kit by kit basis) are run to measure accuracy and precision.

- Qualification – Testing on 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 CVs of less than 20%. CVs and accuracy of the controls are measured on all runs and must meet the kit specification as defined in the Certificate of Analysis (C of A). The typical calculated concentration CV specification is less than 20% and accuracy within 20% of expected concentration and a total error of less than 30%.
- Verification – A multi-day (2-3 days), multiple runs per day for a total of 6-12 plates is performed as part of the release testing for each lot. The specifications for release are provided in the C of A.

➤ 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 C of A for all kit lots. Presented below are representative data from the assay qualification for this assay that meets the criteria described above. The actual kit-specific standard curve and measured limits of quantification can be found in the C of A enclosed with the kit.

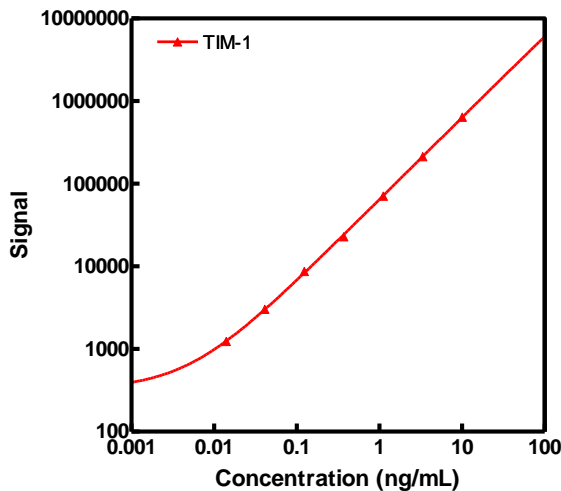
XI Typical Standard Curve

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 quantification of unknown samples.

Some variation in the concentration of the highest Calibrator is permissible between kit lots. Below is a table that details the acceptable range of the highest Calibrator concentration. For each individual kit lot, the Calibrator concentrations are shown in the C of A.

	Highest Calibrator Concentration	
	Target	Acceptable range
TIM-1 (ng/mL)	10	8.50 – 11.5



Conc. (ng/mL)	TIM-1	
	Average Signal	%CV
0	259	5.1
0.0137	1237	2.7
0.0412	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

XII 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 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

XIII 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 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	Plates	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

XIV Spike Recovery

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 x 100

		TIM-1		
Sample	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

XV Linearity

linearity

To assess linearity, 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 x dilution factor) / expected x 100

		TIM-1		
Sample	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

XVI Samples

s a m p l e s

Urine samples collected from normal Sprague-Dawley rats were tested at 10-fold dilution on the Rat TIM-1/KIM-1/HAVCR Assay. Shown below are the median and range of concentrations for each sample set. Concentrations have been corrected for sample dilution.

Sample	Statistic	Anayte
Urine	Median (ng/mL)	1.50
	Range (ng/mL)	0.708 – 4.37
	N	6

XVII Assay Components

a s s a y c o m p o n e n t s

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

Analyte	Source Species	
	MSD Capture Antibody	MSD Detection Antibody
	Goat Polyclonal	Goat Polyclonal

XVIII References

r e f e r e n c e s

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. (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 all reagents to room temperature and thaw the Calibrator on ice.

Prepare an 8-point standard curve using supplied Calibrator:

- The Calibrator should be diluted in Diluent 5.
- Dilute the stock Calibrator 20-fold in Diluent 5 then perform a series of 3-fold dilution steps and a no Calibrator blank.
- If necessary, Dilute samples by 5-fold into Diluent 5 prior to addition to the plate.

Prepare Detection Antibody Solution by diluting the 50X Detection Antibody to 1X in a final volume of 3.0 mL Diluent 5 per plate.

Prepare 20 mL of 1X Read Buffer T by diluting 4X Read Buffer T 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 3 times with PBS-T.

Dispense 25 μ L/well Diluent 5.

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 3 times 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 3 times with PBS-T.

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

Analyze plate on SECTOR instrument.

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