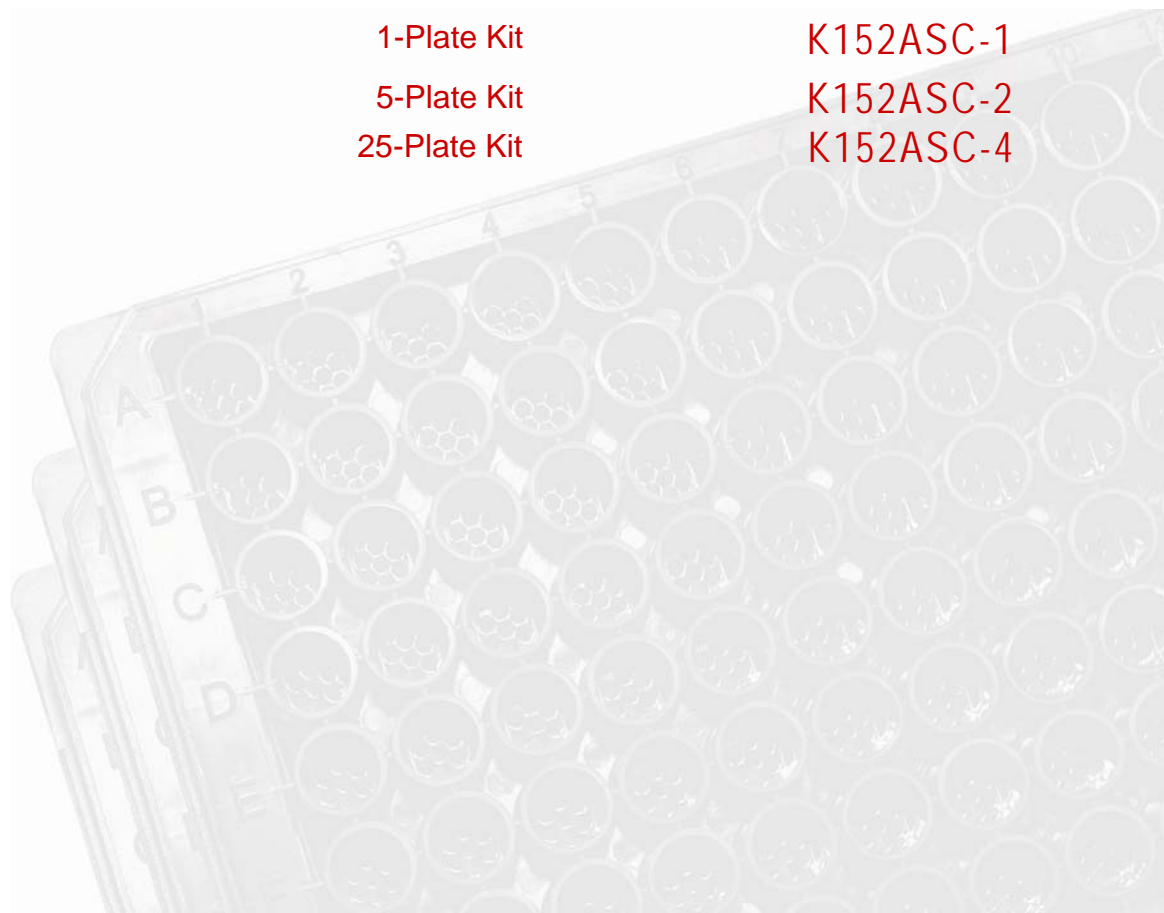


Meso Scale Discovery[®]

MULTI-ARRAY[®] Assay System

Mouse IL-13 Assay
Ultra-Sensitive Kit

1-Plate Kit	K152ASC-1
5-Plate Kit	K152ASC-2
25-Plate Kit	K152ASC-4



Meso Scale Discovery Meso Scale Discovery Meso Scale Discovery Meso Scale Discovery Meso Scale Discovery Meso Scale Discovery Meso Scale Di



MSD MULTI-ARRAY Assay

Ultra-Sensitive Kit

Mouse IL-13 Assay

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.

Meso Scale Discovery, Meso Scale Diagnostics, www.mesoscale.com, MSD, MSD (design), Discovery Workbench, Quickplex, Multi-Array, Multi-Spot, Sulfo-Tag and Sector are trademarks of Meso Scale Diagnostics, LLC.
© 2009 Meso Scale Discovery a division of Meso Scale Diagnostics, LLC. All rights reserved.

Table of Contents

table of contents

Introduction.....	4
Principle of the Assay	5
Reagents Supplied	6
Required Material and Equipment – not supplied.....	6
Safety	6
Reagent Preparation	7
Assay Protocol.....	9
Analysis of Results	9
Typical Standard Curve	10
Sensitivity	10
Spike Recovery	11
Linearity	11
Samples.....	12
References	12
Summary Protocol	13
Plate Diagrams	15

Ordering Information

Ordering information

MSD Customer Service

Phone: 1-301-947-2085

Fax: 1-301-990-2776

Email: CustomerService@mesoscale.com

Meso Scale Discovery

A division of Meso Scale Diagnostics, LLC.

9238 Gaither Road

Gaithersburg, MD 20877 USA

www.mesoscale.com

Introduction

introduction

Interleukin-13 (IL-13) is the latest identified cytokine to join the Th2 cytokine family. Initially identified by screening mouse cDNA libraries for inducible genes,^[1] mouse IL-13 cDNA encodes a 131-amino acid protein that is secreted as an approximately 14-kDa protein by transfected COS-7 and BW5147 cells.^[2] IL-13 belongs to the same protein family as IL-4, but they share limited protein sequence homology of 30%.^[3,4] Structurally, however, IL-13 is very similar to IL-4.^[5] The receptor for IL-13 is comprised of the IL-4 receptor alpha (IL-4R α) subunit and a low-affinity IL-13-binding subunit, IL-13R α 1. An additional receptor, IL-13R α 2, binds to IL-13 with high affinity, but lacks the cytoplasmic domain for signaling. Due to their protein structure homology and common receptors, the biological functions of IL-13 partially overlap with those of IL-4.^[6]

Interleukin-13 has been implicated as a key factor in asthma, allergy, atopy and inflammatory response.^[5,7] IL-13 (in common with IL-4) induces morphological and surface antigen changes in human monocytes and mouse granulocyte-macrophage CSF (GM-CSF) derived bone marrow macrophages. IL-13 also inhibits antibody-dependent cellular cytotoxicity (ADCC) activity and production of nitric oxide and proinflammatory cytokines^[8,9,10,11] without affecting the phagocytic functions of activated macrophages.^[11] IL-13 administration in vivo induces extramedullary hemopoiesis and monocytosis in mice.^[2] Additionally, IL-13R α 2 chain is overexpressed in many human cancer cell lines and primary cell cultures including glioblastoma, AIDS-associated Kaposi's sarcoma, ovarian carcinoma, renal cell carcinoma and fibroblast cells.^[12] Due to its essential role in several disease conditions, IL-13 serves as a valuable therapeutic target and biomarker.

Principle of the Assay

principle of the assay

MSD[®] 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. The antibody for a specific protein target is coated on one electrode (or “spot”) per well. The Mouse IL-13 Assay detects IL-13 in a sandwich immunoassay format (Figure 1). For this assay, MSD provides a Cytokine Panel 15 plate that has been pre-coated with capture antibodies on spatially distinct spots. The position of IL-13 capture antibody is indicated in Figure 1 and on the plate packaging. The user adds the sample and a solution containing the labeled detection antibody— anti-IL-13 labeled with an electrochemiluminescent compound, MSD SULFO-TAG[™] label—over the course of one or more incubation periods. IL-13 in the sample binds to capture antibody immobilized on the working electrode surface; recruitment of the labeled detection antibody by bound IL-13 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 IL-13 present in the sample.

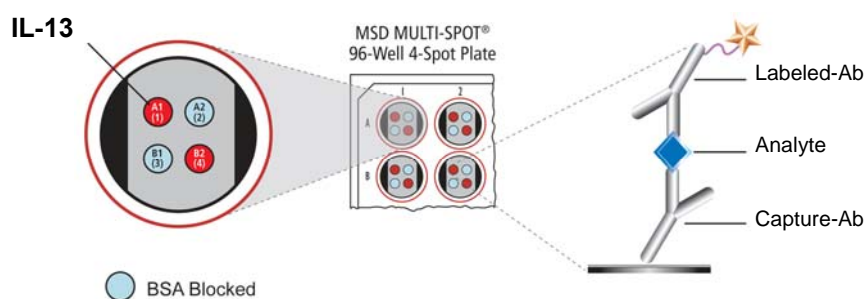


Figure 1. Spot diagram showing placement of analyte capture antibody on Cytokine Panel 15 Plate. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. Any spot that is not coated with a specific capture antibody is blocked with BSA to reduce non-specific binding to that spot. 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		
		K152ASC-1	K152ASC-2	K152ASC-4
MULTI-SPOT® 96-well 4 Spot Cytokine Panel 15 Plate(s) N45037B-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG™ Anti-mIL-13 Antibody ¹ (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Mouse IL-13 Calibrator (10 µg/mL)	≤-70°C	1 vial (15 µL)	5 vials (15 µL ea)	25 vials (15 µL ea)
Diluent 4 R52BB-4 (8 mL) R52BB-3 (40 mL)	≤-10°C	1 bottle (8 mL)	1 bottle (40 mL)	5 bottles (40 mL ea)
Diluent 5 R52BA-4 (5 mL) R52BA-5 (25 mL)	≤-10°C	1 bottle (5 mL)	1 bottle (25 mL)	5 bottles (25 mL ea)
Read Buffer T (4X) R92TC-3 (50 mL) R92TC-2 (200 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	2 bottles (200 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 and thaw the Calibrator stock on ice.

Important: Upon first thaw, separate Diluent 4 and 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 Calibrator and Control Solutions

MSD recommends the preparation of an 8-point standard curve consisting of at least 2 replicates of each point. Each well requires 25 μ L of Calibrator. For the assay, MSD recommends 4-fold serial dilution steps and Diluent 4 alone for the 8th point:

Standard	IL-13 (pg/mL)	Dilution Factor
100X Stock	10000000	
STD-01	100000	100
STD-02	25000	4
STD-03	6250	4
STD-04	1560	4
STD-05	390	4
STD-06	98	4
STD-07	24	4
STD-08	0	n/a

To prepare this 8-point standard curve for up to 4 replicates:

- 1) Prepare the highest Calibrator point (STD-01) by transferring 10 μ L of the Mouse IL-13 Calibrator Blend to 990 μ L Diluent 4.
- 2) Prepare the next Calibrator by transferring 50 μ L of the diluted Calibrator to 150 μ L of Diluent 4. Repeat 4-fold serial dilutions 5 additional times to generate 7 Calibrators.
- 3) The recommended 8th Standard is Diluent 4 (i.e. zero Calibrator).

Notes:

- a. Alternatively, Calibrators can be prepared in the sample matrix or diluent of choice to verify acceptable performance in these matrices. In general, the presence of some protein (for example, 1% BSA) in the sample matrix is helpful for preventing loss of analyte by adsorption onto the sides of tubes, pipette tips, and other surfaces. If your sample matrix is serum-free tissue culture media, then the addition of 10% FBS or 1% BSA is recommended.
- b. The standard curve can be modified as necessary to meet specific assay requirements.

Dilution of Samples

Serum and Plasma

All solid material should be removed by centrifugation. Plasma prepared in heparin tubes commonly displays additional clotting following the thawing of the sample. Remove any additional clotted material by centrifugation. Avoid multiple freeze/thaw cycles for serum and plasma samples. Serum and plasma samples may be run neat in the MSD Mouse IL-13 Assay.

Tissue Culture

Tissue culture supernatant samples may not require dilution prior to being used in the MSD Mouse IL-13 Assay. If using serum-free medium, the presence of carrier protein (e.g., 1% BSA) in the solution is helpful to prevent loss of analyte to the labware. Samples from experimental conditions with extremely high levels of cytokines may require a dilution.

Other Matrices

Information on preparing samples in other matrices, including sputum, CSF, and tissue homogenates can be obtained by contacting MSD Scientific Support at 1-301-947-2025 or ScientificSupport@mesoscale.com.

Prepare Detection Antibody Solution

The Detection Antibody is provided at 50X stock of Anti-mIL-13 Antibody. The final concentration of the working Detection Antibody Solution should be at 1X. For each plate used, dilute a 60 μ L aliquot of the stock Anti-mIL-13 Antibody into 2.94 mL of Diluent 5.

Prepare Read Buffer

The Read Buffer should be diluted 2-fold in deionized water to make a final concentration of 2X Read Buffer T. Add 10 mL of 4X Read Buffer T to 10 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.

VII Assay Protocol

assay protocol

- 1. Addition of Diluent 4:** Dispense 25 μL of Diluent 4 into each well. Seal the plate with an adhesive plate seal and incubate for 30 min with vigorous shaking (300–1000 rpm) at room temperature.
- 2. Addition of the Sample or Calibrator:** Dispense 25 μL of 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 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 2X 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 plate typically accelerates capture at the working electrode.

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

VIII Analysis of Results

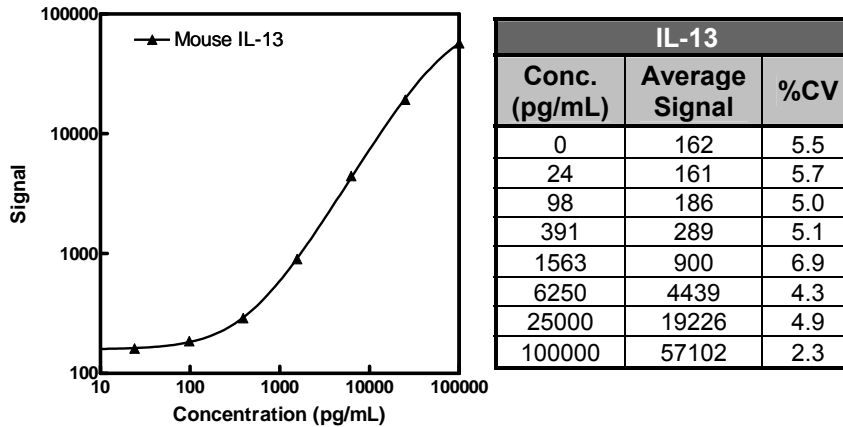
analysis of results

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 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.

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.



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. The value below represents the average LLOD over multiple kit lots.

IL-13	
LLOD (pg/mL)	139

XI Spike Recovery

spike recovery

Mouse serum, EDTA plasma and heparin plasma pooled samples were spiked with Calibrator at multiple values throughout the range of the assay. Each spike was done in ≥ 3 replicates. An average of two serum and three EDTA plasma are shown here. Results of spike-recovery may vary based on the individual samples.

$\% \text{ Recovery} = \text{measured} / \text{expected} \times 100$

Sample	Spike Conc. (pg/mL)	Measured Conc. (pg/mL)	Measured Conc. % CV	% Recovery
Serum	0	0	10.1	
	156	158	3.2	101
	625	596	4.3	95
	2500	1248	2.8	50
EDTA Plasma	0	453	9.3	
	156	544	0.3	89
	625	878	1.5	81
	2500	2016	1.6	68
Heparin Plasma	0	233	8.4	
	156	407	4.2	105
	625	885	2.7	103
	2500	1605	3.3	59

XII Linearity

linearity

Three pools each of mouse serum and heparin plasma were evaluated; a representative pool of each is shown below. The pooled samples were spiked with Calibrator and then diluted with Diluent 4. 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 of the previous dilution (expected).

$\% \text{ Recovery} = (\text{measured} \times \text{dilution factor}) / \text{expected} \times 100$

Sample	Fold Dilution	Conc. (pg/mL)	Conc. % CV	% Recovery
Serum	1	4605	5.8	
	2	4495	2.3	98
	4	4464	2.8	99
	8	4513	5.5	101
Heparin Plasma	1	4207	8.6	
	2	4562	3.5	108
	4	5140	8.5	113
	8	5272	2.3	103

XIII Samples

s a m p l e s

Eight normal mouse serum samples were measured and the endogenous levels were found to be below LLOD.

XIV References

r e f e r e n c e s

1. Brown KD, Zurawski SM, Mosmann TR, Zurawski G. A family of small inducible proteins secreted by leukocytes are members of a new superfamily that includes leukocyte and fibroblast-derived inflammatory agents, growth factors, and indicators of various activation processes. *J Immunol.* 1989 Jan 15;142(2):679-87.
2. Lai YH, Heslan JM, Poppema S, Elliott JF, Mosmann TR. Continuous administration of IL-13 to mice induces extramedullary hemopoiesis and monocytosis. *J Immunol.* 1996 May 1;156(9):3166-73.
3. Zurawski G, de Vries JE. Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. *Immunol Today.* 1994 Jan;15(1):19-26.
4. Zurawski SM, Vega F Jr, Huyghe B, Zurawski G. Receptors for interleukin-13 and interleukin-4 are complex and share a novel component that functions in signal transduction. *EMBO J.* 1993 Jul;12(7):2663-70.
5. Moy FJ, Diblasio E, Wilhelm J, Powers R. Solution structure of human IL-13 and implication for receptor binding. *J Mol Biol.* 2001 Jun 29;310(1):219-30.
6. Osawa M, Miyoshi S, Copeland NG, Gilbert DJ, Jenkins NA, Hiroyama T, Motohashi T, Nakamura Y, Iwama A, Nakauchi H. Characterization of the mouse interleukin-13 receptor alpha1 gene. *Immunogenetics.* 2000 Sep;51(11):974-81.
7. Doucet C, Brouty-Boyé D, Pottin-Clémenceau C, Canonica GW, Jasmin C, Azzarone B. Interleukin (IL) 4 and IL-13 Act on Human Lung Fibroblasts. Implication in Asthma. *J Clin Invest.* 1998 May 15;101(10):2129-39.
8. Minty A, Chalon P, Derocq JM, Dumont X, Guillemot JC, Kaghad M, Labit C, Leplatois P, Liauzun P, Miloux B, et al. Interleukin-13 is a new human lymphokine regulating inflammatory and immune responses. *Nature.* 1993 Mar 18;362(6417):248-50.
9. McKenzie AN, Culpepper JA, de Waal Malefyt R, Brière F, Punnonen J, Aversa G, Sato A, Dang W, Cocks BG, Menon S, et al. Interleukin 13, a T-cell-derived cytokine that regulates human monocyte and B-cell function. *Proc Natl Acad Sci U S A.* 1993 Apr 15;90(8):3735-9.
10. de Waal Malefyt R, Figdor CG, Huijbens R, Mohan-Peterson S, Bennett B, Culpepper J, Dang W, Zurawski G, de Vries JE. Effects of IL-13 on phenotype, cytokine production, and cytotoxic function of human monocytes: comparison with IL-4 and modulation by IFN- or IL-10. *J Immunol.* 1993 Dec 1;151(11):6370-81
11. Doherty TM, Kastelein R, Menon S, Andrade S, Coffman RL. Modulation of murine macrophage function by IL-13. *J Immunol.* 1993 Dec 15;151(12):7151-60
12. Joshi BH, Plautz GE, Puri RK. IL-13 receptor alpha chain: a novel tumor associated transmembrane protein in primary explants of human malignant gliomas. *Cancer Res.* 2000 Mar 1;60(5):1168-72.

Summary Protocol

MSD 96-well MULTI-ARRAY Mouse IL-13 Assay: Ultra-Sensitive Kit

MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the MSD Mouse IL-13 Assay.

Step 1 : Sample and Reagent Preparation

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

Samples may not require dilution prior to use in this assay.

Prepare calibrator solutions and standard curve.

Use the 10 µg/mL Calibrator stock to prepare an 8-point standard curve by diluting in Diluent 4.

Note: *The standard curve can be modified as necessary to meet specific assay requirements.*

Prepare Detection Antibody Solution by diluting Detection Antibody to 1X in 3.0 mL of Diluent 5 (per plate).

Prepare 20 mL of 2X Read Buffer T by diluting 4X MSD Read Buffer T with deionized water.

SERUM OR PLASMA SAMPLES

Step 2: Add Diluent 4

Dispense 25 µL/well Diluent 4.

Incubate at room temperature with vigorous shaking (300-1000 rpm) for 30 minutes.

Step 3: Add Sample or Calibrator

Dispense 25 µL/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 µL/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 µL/well 2X Read Buffer T.

Analyze plate on SECTOR Imager instrument.

