MSD® MULTI-SPOT Assay System

Human Eotaxin-2 Kit

1-Plate Kit	K151RKD-1
5-Plate Kit	K151RKD-2
25-Plate Kit	K151RKD-4

MSD Cytokine Assays

Human Eotaxin-2 Kit

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

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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A division of Meso Scale Diagnostics, LLC.
1601 Research Boulevard
Rockville, MD 20850-3173 USA
www.mesoscale.com

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Ordering Information

MSD Customer Service

Phone: 1-301-947-2085
Fax: 1-301-990-2776
Email: CustomerService@mesoscale.com

MSD Scientific Support

Phone: 1-301-947-2025
Fax: 1-240-632-2219 attn: Scientific Support
Email: ScientificSupport@mesoscale.com

Introduction

Eotaxin-2 (CCL24) is a C-C chemokine attractant for eosinophils, basophils, and Th2 lymphocytes.[1,2] As part of the eotaxin family, eotaxin-2 is specific for receptor CCR3, which also binds ligands RANTES, MCP-3, and MCP-4.[1] Eotaxin and eotaxin-2 are both strongly attractive to eosinophils and are likely critical in eosinophilic recruitment, activation, and degranulation.[2,3]

Eotaxin-2's chemotactic effect on eosinophils implicates it in allergic reactions and asthma[2-4] but does not preclude its involvement in other immune responses, such as arthritis,[1] or in colorectal cancer.[5] Localized eosinophil accumulations are directly correlated to eotaxin-2 dosage concentration[3,4] and are independent of the allergic status of the subject.[3] Eotaxin-2 expression is also found in abundance in primary colorectal cancer and colorectal liver metastases, which when compared to normal tissue expression, hints at a role in tumor environment priming and conditioning.[5]

Principle of the Assay

MSD cytokine assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. Human Eotaxin-2 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 a SECTOR® Imager 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.

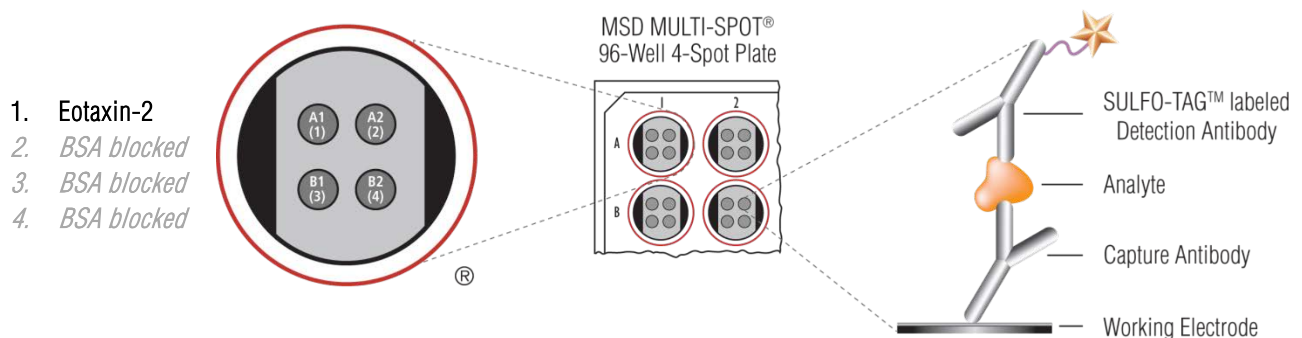


Figure 1. Spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.

Reagents Supplied

Product Description	Storage	Quantity per Kit K151RKD-1	K151RKD-2	K151RKD-4
MULTI-SPOT 96-Well 4-Spot Human Eotaxin-2 Plate N451RKA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-hu Eotaxin-2 Antibody[1] (50X) D21RK-2 (75 µL), D21RK-3 (375 µL)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human Eotaxin-2 Calibrator (0.04 µg/mL) C01RK-2	≤-70°C	1 vial (60 µL)	5 vials (60 µL ea)	25 vials (60 µL ea)
Diluent 7 R54BB-3 (5 mL), R54BB-4 (50 mL)	≤-10°C	1 bottle (5 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)
Diluent 8 R54BA-4 (5 mL), R54BA-3 (50 mL)	≤-10°C	1 bottle (5 mL)	1 bottle (50 mL ea)	5 bottles (50 mL ea)
Read Buffer T (4X) R92TC-3 (50 mL)	RT	1 bottle (50 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)

Required Material and Equipment (not supplied)

- ❑ 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 safety information is available in the product Material Safety Data Sheet, 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 7 and Diluent 8 into aliquots appropriate for the size of your needs before refreezing.

Prepare Standards

MSD supplies calibrator for the Human Eotaxin-2 Kit at 20-fold higher concentration than the recommended highest standard. We recommend a 7-point standard curve with 4-fold serial dilution steps and a zero calibrator blank. Signals from the blank should be excluded when generating the curve. Thaw the stock calibrator, then add to diluent at room temperature to make the standard curve solutions.

Standard	Human Eotaxin-2(pg/mL)	Dilution Factor
Stock Calibrator	40 000	
STD-01	2000	20
STD-02	500	4
STD-03	125	4
STD-04	31	4
STD-05	7.8	4
STD-06	2.0	4
STD-07	0.49	4
STD-08	0	n/a

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

1) Prepare the highest standard by adding 50 µL of stock calibrator to 950 µL of Diluent 7. Mix well.
2) Prepare the next standard by transferring 100 µL of the highest standard to 300 µL of Diluent 7 Mix well. Repeat 4-fold serial dilutions 5 additional times to generate 7 standards.
3) Use Diluent 7 as the blank.

Dilute Samples

Serum and Plasma

Plasma prepared in heparin tubes commonly displays additional clotting following thawing of the sample. Remove clots and all solid material by centrifugation. Avoid multiple freeze–thaw cycles for serum and plasma samples. Dilute serum and plasma samples at least 10-fold in Diluent 7; however, you may adjust dilution factors for the sample set under investigation.

Tissue Culture

If using a 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. Dilute tissue culture supernatant samples at least 10-fold in Diluent 7. Samples with extremely high levels of cytokines may require additional dilution.

Prepare Detection Antibody Solution

MSD provides each detection antibody in a 50X stock solution. The working detection antibody solution is 1X. Avoid exposing 1X detection antibody solution to light to prevent elevated background signals.

For 1 plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-hu Eotaxin-2 Antibody
- ❑ 2940 µL of Diluent 8

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 2X.

For 1 plate, combine:

- ❑ 10 mL of Read Buffer T (4X)
- ❑ 10 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 Sample or Calibrator:** Add 50 µL of sample (standards, controls, 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.

2. **Wash and Add Detection Antibody Solution:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 25 µL of 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.

3. **Wash and Read:** Wash the plate 3 times with 300 µL/well of PBS-T. Add 150 µL of 2X Read Buffer T to each well. Analyze the plate on the SECTOR Imager. 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.

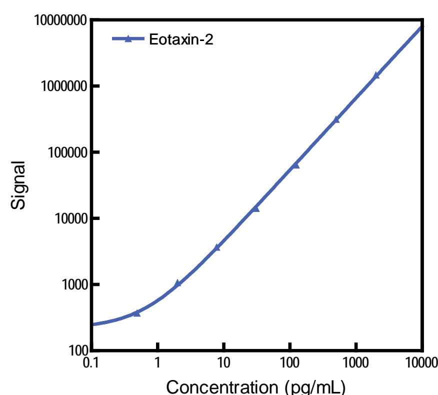
Due to the varying nature of each research application, you should assess assay stability before allowing plates to sit with read buffer for extended periods.

Curve Fitting

MSD DISCOVERY WORKBENCH® software uses least-squares fitting algorithms to generate the standard curve that will be used to calculate the concentration of analyte in the samples. The assays have a wide dynamic range (3–4 logs) that allows accurate quantification without the need for dilution in many cases. By default, the software uses 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.

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.



Eotaxin-2		
Conc. (pg/mL)	Average Signal	%CV
0	133	10.8
0.49	370	3.6
2.0	1068	7.9
7.8	3681	7.3
31	14 128	3.9
125	64 309	5.2
500	315 587	5.6
2000	1 475 581	6.6

Sensitivity

The lower limit of detection (LLOD) is a calculated concentration based on a signal 2.5 standard deviations above the background (zero calibrator blank).

	Eotaxin-2
Average LLOD (pg/mL)	0.10

Assay Components

Calibrator

The assay calibrator uses recombinant human Eotaxin-2, (residues 27–119), expressed in *E.coli*.

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MDS Detection Antibody
Eotaxin-2	Mouse Monoclonal	Goat Polyclonal

References

1. Ablin JN, et al. Protective effect of Eotaxin-2 inhibition in adjuvant-induced arthritis. Clin Exp Immunol. 2010 Aug;161(2):276-83.
2. Ochkur SI, et al. Coexpression of IL-5 and Eotaxin-2 in mice creates an eosinophil-dependent model of respiratory inflammation with characteristics of severe asthma. J Immunol. 2007 Jun 15;178(12):7879-89.
3. Menzies-Gow A, et al. Eotaxin (CCL11) and Eotaxin-2 (CCL24) induce recruitment of eosinophils, basophils, neutrophils, and macrophages as well as features of early- and late-phase allergic reactions following cutaneous injection in human atopic and nonatopic volunteers. J Immunol. 2002 Sep 1;169(5):2712-8.
4. Olze H, et al. Eosinophilic nasal polyps are a rich source of Eotaxin, Eotaxin-2, and Eotaxin-3. Rhinology. 2006 Jun;44(2):145-50.
5. Cheadle EJ, et al. Eotaxin-2 and colorectal cancer: a potential taret for immune therapy. Clin Cancer Res. 2007 Oct 1;13(19):5719-28.

Summary Protocol

Human Eotaxin-2 Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Human Eotaxin-2 assays.

Sample and Reagent Preparation

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

Prepare standard solutions using the supplied calibrator:

- Dilute the stock calibrator 20-fold in Diluent 7.
- Perform a series of 4-fold dilution steps and prepare a zero calibrator blank.

Dilute samples 10-fold in Diluent 7 before adding to the plate.

Prepare detection antibody solution by diluting stock detection antibody 50-fold in Diluent 8.

Prepare 2X Read Buffer T by diluting stock 4X Read Buffer T 2-fold with deionized water.

Step 1: Add Sample

Add 50 µL/well of sample (standards, controls, or unknowns).

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

Step 2: 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 3: Wash and Read Plate

Wash plate 3 times with 300 µL/well of PBS-T.

Add 150 µL/well of 2X Read Buffer T.

Analyze plate on SECTOR Imager.

Plate Diagrams