

MSD MULTI-ARRAY[®] Assay System

Human Fractalkine Kit

1-Plate Kit	K151MKD-1
5-Plate Kit	K151MKD-2
25-Plate Kit	K151MKD-4



MSD[®] Cytokine Assays

Human Fractalkine 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[®]

A division of Meso Scale Diagnostics, LLC.

9238 Gaither Road

Gaithersburg, MD 20877 USA

www.mesoscale.com

MESO SCALE DISCOVERY, MESO SCALE DIAGNOSTICS, WWW.MESOSCALE.COM, MSD, MSD (DESIGN), DISCOVERY WORKBENCH, QUICKPLEX, MULTI-ARRAY, MULTI-SPOT, SULFO-TAG, SECTOR, SECTOR HTS, SECTOR PR, 4-SPOT (DESIGN), and SPOT THE DIFFERENCE are trademarks and/or service marks of Meso Scale Diagnostics, LLC.

© 2012 Meso Scale Diagnostics, LLC. All rights reserved

Table of Contents

Introduction	4
Principle of the Assay	4
Reagents Supplied	5
Required Material and Equipment (not supplied)	5
Safety	5
Reagent Preparation.....	6
Assay Protocol.....	8
Analysis of Results	8
Typical Data.....	9
Sensitivity.....	9
Assay Components	10
References.....	10
Summary Protocol.....	11
Plate Diagrams	13

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

Fractalkine (CX3CL1) is a multi-functional, transmembrane protein with an extracellular, chemokine domain that sits atop a mucin-like stalk which aids in the binding of certain immune cells. The transmembrane form can be proteolytically processed to generate a secreted 90 kDa soluble form.¹ Soluble fractalkine has been observed in serum, urine, CSF, and synovial fluids. The soluble form acts as a chemoattractant for monocytes, natural killer (NK) cells, and lymphocytes. Fractalkine is thought to mediate interactions between neurons and microglia in the brain, and protects the brain against reactive oxygen species during neuroinflammation.²

The membrane-bound form is predominantly expressed on vascular endothelium, smooth muscle, neurons and epithelial linings of the intestine, bronchi, and renal proximal tubules. At these surfaces, TNF- α , IFN- γ , and IL-1 promote increased fractalkine expression and subsequent adhesion of leukocytes and extracellular matrix proteins to the inflamed endothelial cells.¹⁻³ Thus, fractalkine is involved in the pathogenesis of various clinical disease states or processes, such as atherosclerosis, glomerulonephritis, renal and cardiac allograft rejection, and rheumatoid arthritis.²⁻⁴

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. The Human Fractalkine Assay 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 intensity of emitted light to provide a quantitative measure of analytes in the sample.

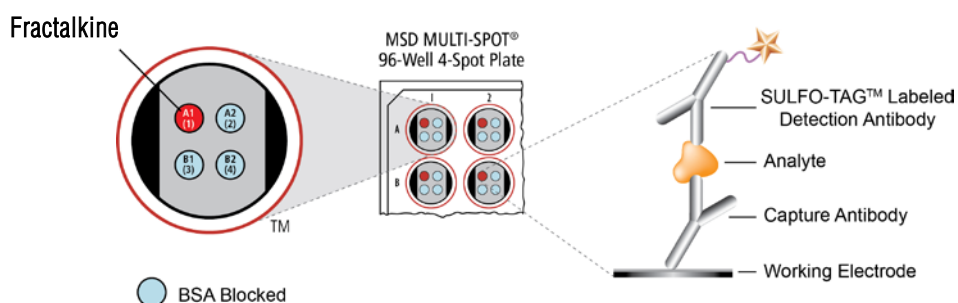


Figure 1. Spot diagram showing placement of analyte capture antibody. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing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

Reagents Supplied

Product Description	Storage	Quantity per Kit		
		K151MKD-1	K151MKD-2	K151MKD-4
MULTI-SPOT 96-Well 4-Spot Human Fractalkine Plate N451MKA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-hu Fractalkine Antibody ¹ (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human Fractalkine Calibrator (10 µg/mL)	≤-70°C	1 vial (15 µL)	5 vials (15 µL ea)	25 vials (15 µL ea)
Diluent 43 R50AG-1 (8 mL), R50AG-2 (40 mL)	≤-10°C	1 bottle (8 mL)	1 bottle (40 mL)	5 bottles (40 mL ea)
Diluent 3 R51BA-4 (5 mL), R51BA-5 (25 mL)	≤-10°C	1 bottle (5 mL)	1 bottle (25 mL)	5 bottles (25 mL ea)
Blocker A Kit R93AA-2 (250 mL)	RT	1 bottle (250 mL)	1 bottle (250 mL)	5 bottles (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)

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

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.

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 43 and Diluent 3 into aliquots appropriate to the size of your assay needs. These diluents can go through 3 freeze-thaw cycles without significantly affecting the performance of the assay.

Prepare Blocker A Solution

Follow instructions included with the Blocker A Kit.

Prepare Standards

MSD recommends an 8-point standard curve with 4-fold serial dilution steps and a zero calibrator. Thaw the stock calibrator and keep on ice, then add to diluent at room temperature to make the standard curve solutions.

Standard	Fractalkine Calibrator (pg/mL)	Dilution Factor
Stock Calibrator	10 000 000	
STD-01	100 000	100
STD-02	25 000	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 8 standard solutions for up to 3 replicates:

- 1) Prepare the highest standard by adding 10 μ L of the stock calibrator to 990 μ L of Diluent 43. Mix well.
- 2) Prepare the next standard by transferring 75 μ L of the highest standard to 225 μ L of Diluent 43. Mix Well. Repeat 4-fold serial dilutions 5 additional times to generate 7 standards.
- 3) Use Diluent 43 as the 8th standard (i.e. zero calibrator).

Notes

- a. Alternatively, standards may be prepared in the sample matrix or diluent of choice. 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.
- b. You may modify the standard solutions as necessary to meet specific assay requirements.

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 2-fold in Diluent 43.

Tissue Culture

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 with extremely high levels of cytokines may require dilution. Dilute tissue culture supernatant samples at least 2-fold in Diluent 43.

Other Matrices

For information on preparing samples in other matrices, such as sputum, CSF, and tissue homogenates, contact MSD Scientific Support at 1-301-947-2025 or ScientificSupport@mesoscale.com

Prepare Detection Antibody Solution

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

For 1 plate, combine:

- 60 μ L of 50X SULFO-TAG Anti-hu Fractalkine Antibody
- 2.94 mL of Diluent 3

Prepare Read Buffer

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

For 1 plate, combine:

- 10 mL Read Buffer T (4X)
- 10 mL 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 (see 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.

Assay 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 or Calibrator:** Wash the plate 3 times with 300 μL /well of PBS-T. Add 50 μL of calibrator or diluted sample 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 into each well 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.
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 2X Read Buffer T to each well of the MSD plate. Analyze the plate on the SECTOR Imager. No incubation in read buffer is required.

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.

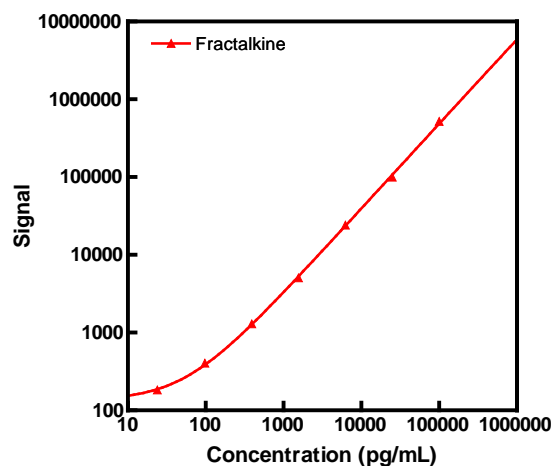
Due to the varying nature of each research application, you should assess stability before allowing plates to sit with read buffer for extended periods.

Analysis of Results

MSD DISCOVERY WORKBENCH[®] software uses least-squares fitting algorithms to generate a 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) which allows accurate quantification without the need for dilution in many cases. 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 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.



Fractalkine		
Conc. (pg/mL)	Average Signal	%CV
0	112	4.4
24	183	3.5
98	405	3.1
390	1295	1.4
1560	5057	4.1
6250	24 039	7.5
25 000	99 715	12.6
100 000	517 917	1.1

Sensitivity

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

Fractalkine	
LLOD (pg/mL)	16

Assay Components

Calibrator

The assay calibrator uses recombinant human Fractalkine protein expressed in NSO-derived murine myeloma cell line.

Antibodies

Analyte	Source Species	
	MSD Capture Antibody	MSD Detection Antibody
Fractalkine	Mouse Monoclonal	Goat Polyclonal

References

1. Umehara H, Bloom ET, Okazaki T, Nagano Y, Yoshie O, Imai T. Fractalkine in vascular biology: from basic research to clinical disease. *Arterioscler Thromb Vasc Biol.* 2004 Jan;24(1):34-40.
2. Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. *N Engl J Med.* 2006 Feb 9;354(6):610-21.
3. Lee DW, Faubel S, Edelstein CL. Cytokines in acute kidney injury (AKI). *Clin Nephrol.* 2011 Sep;76(3):165-73.
4. Jones BA, Beamer M, Ahmed S. Fractalkine/CX3CL1: a potential new target for inflammatory diseases. *Mol Interv.* 2010 Oct;10(5):263-70.

Summary Protocol

MSD 96-well Human Fractalkine Kit

*MSD provides this summary protocol for your convenience.
Please read the entire detailed protocol prior to performing the
Human Fractalkine assay.*

Sample and Reagent Preparation

Bring all reagents to room temperature, and thaw the calibrator on ice.
Prepare Blocker A solution.
Prepare 8 standard solutions using the supplied calibrator as described in the “Prepare Standards” section.
Dilute samples 2-fold in Diluent 43 before adding to the plate.
Prepare detection antibody solution by diluting 50X detection antibody 50-fold in Diluent 3.
Prepare 2X Read Buffer T by diluting 4X Read Buffer T 2-fold with deionized water.

Step 1: Add Blocker A Solution

Add 150 μ L/well of Blocker A solution.
Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 2: Wash and Add Sample or Calibrator

Wash plate 3 times with 300 μ L/well of PBS-T.
Add 50 μ L/well of calibrator or diluted sample.
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 μ L/well of PBS-T.
Add 25 μ L/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 μ L/well of PBS-T.
Add 150 μ L/well of 2X Read Buffer T.
Analyze plate on SECTOR Imager.

	1	2	3	4	5	6	7	8	9	10	11	12
A	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
B	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
C	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
D	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
F	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
G	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
H	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

	1	2	3	4	5	6	7	8	9	10	11	12
A	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
B	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
C	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
D	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
E	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
F	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
G	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
H	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>