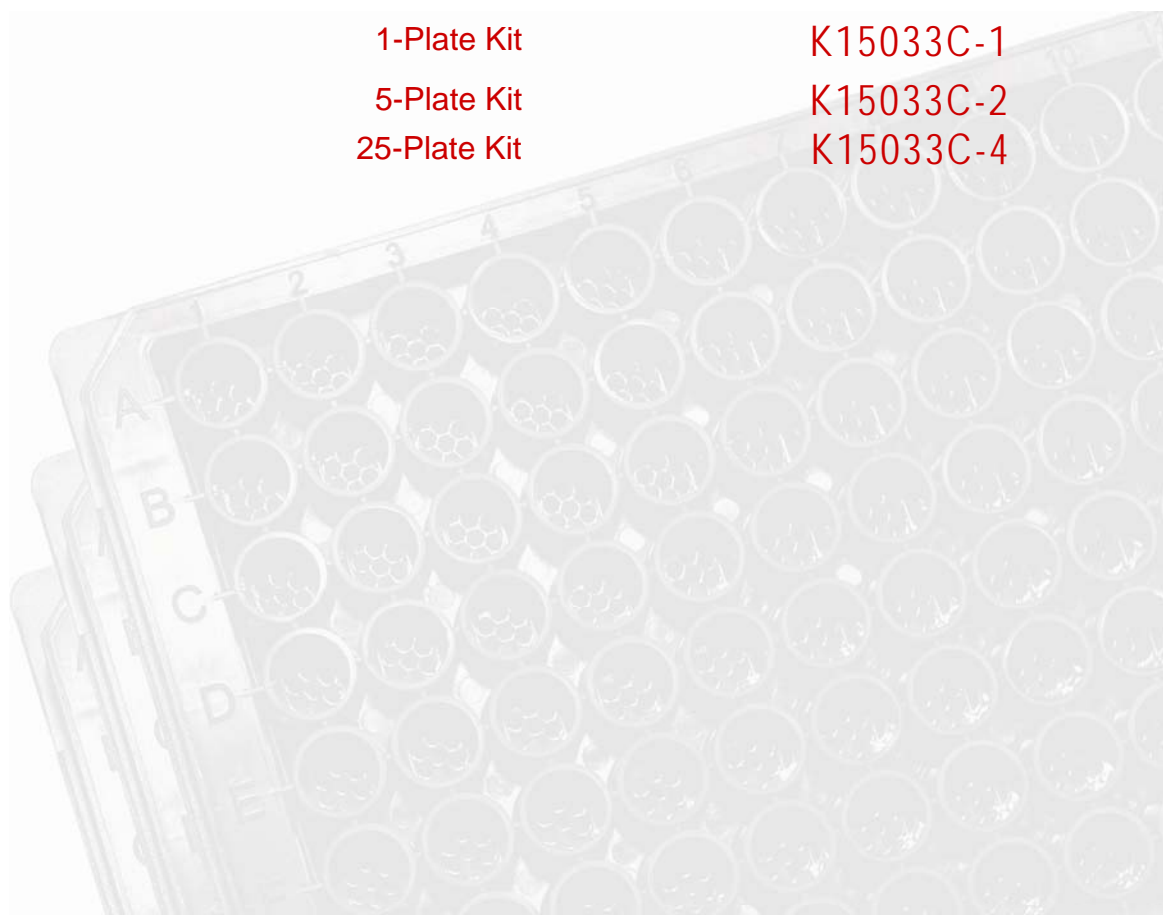


Meso Scale Discovery[®]

MULTI-SPOT[®] Assay System

Human MMP 2-Plex Assay
Ultra-Sensitive Kit

1-Plate Kit	K15033C-1
5-Plate Kit	K15033C-2
25-Plate Kit	K15033C-4



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MSD MULTI-SPOT Assays

Ultra-Sensitive Kit

Human MMP 2-Plex Assay

MMP-2, MMP-10

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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Introduction

introduction

Matrix metalloproteinases (MMP) are a family of endopeptidases which are critically important in extracellular matrix remodeling. They are highly homologous to each other and belong to a larger family of proteases known as the metzincin superfamily. The MMP family currently includes more than 25 members that can be divided into collagenases, gelatinases, stromelysins, matrilysins and the membrane-type MMPs. The MMPs are important for several normal functions such as embryonic development, wound healing, etc. They have also been implicated in many pathological conditions such as angiogenesis, inflammation, respiratory, cardiovascular and central nervous diseases.

MMP-2 degrades type IV collagen, which is the major component of basement membranes. It is expressed in fibroblasts during development and tissue regeneration. MMP-2 is tightly regulated at the transcriptional and post-transcriptional levels. MMP-2 in coordination with other MMPs, play a role in normal tissue remodeling events such as embryonic development, angiogenesis, ovulation, mammary gland involution and wound healing. It has also been associated with the ability of tumors to invade and become neovascularized, and is one of the important targets for the development of antitumor therapeutics.

MMP-10, also called Stromelysin-2, is produced as an inactive form and requires proteolytic cleavage for activation. It has the ability to degrade type 3 and type 4 collagens, gelatin, laminin-1, proteoglycans and elastin. MMP-10 is expressed in monocytes and microglial cells. This enzyme is involved in several pathological conditions, such as cancer, arthritis and wound healing.

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. 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. The Human MMP 2-Plex Assay detects MMP-2 and MMP-10 in a sandwich immunoassay format (Figure 1). MSD provides a plate that has been pre-coated with capture antibody on spatially distinct spots – antibodies for MMP-2 and MMP-10. The user adds the sample and a solution containing the labeled detection antibodies—anti-MMP-2 and anti-MMP-10 labeled with an electrochemiluminescent compound, MSD SULFO-TAG[™] label—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 labeled detection antibodies 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 afford a quantitative measure of MMP-2 and MMP-10 present in the sample.

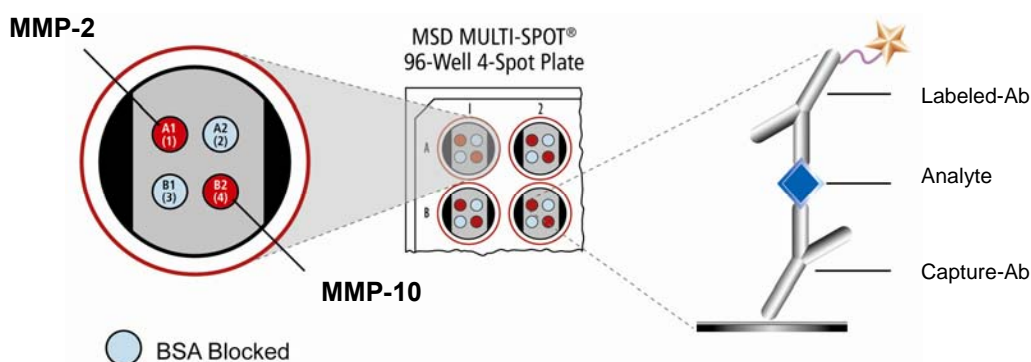


Figure 1. Spot diagram showing placement of analyte capture antibody. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing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



Reagents Supplied

reagents supplied

Product Description	Storage	Quantity per Kit		
		K15033C-1	K15033C-2	K15033C-4
MULTI-SPOT 96-well 4 Spot Human MMP 2-Plex Plate N45033A-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG™ Detection Antibody Blend ¹ (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Human MMP 2-Plex Calibrator Blend (5 µg/mL of MMP-2 and 1 µg/mL of MMP-10)	≤-70°C	1 vial (20 µL)	5 vials (20 µL ea)	10 vials (20 µL ea)
Diluent 2 R51BB-4 (8 mL) R51BB-3 (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)
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 2 and Diluent 3 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

Calibrator for the Human MMP 2-Plex Assay is supplied at 10-fold higher concentration than the recommended highest calibrator. Prepare the highest Calibrator point by diluting the stock Calibrator 10-fold in Diluent 2. 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 2 alone for the 8th point:

Standard	Human MMP 2-Plex Calibrator Blend (pg/mL)		Dilution Factor
	MMP-2	MMP-10	
10X Stock	5000000	1000000	
STD-01	500000	100000	10
STD-02	125000	25000	4
STD-03	31250	6250	4
STD-04	7813	1563	4
STD-05	1953	391	4
STD-06	488	98	4
STD-07	122	24	4
STD-08	0	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 15 μ L of the Human MMP 2-Plex Calibrator Blend to 135 μ L Diluent 2.
- 2) Prepare the STD-02 by transferring 40 μ L of the Human MMP 2-Plex diluted stock Calibrator to 120 μ L Diluent 2. Repeat 4-fold serial dilutions 5 additional times to generate 7 Calibrators.
- 3) The recommended 8th Standard is Diluent 2 (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. Some analytes in this matrix are extremely sensitive to multiple freeze/thaw cycles and the ability to detect these analytes may decrease following the first round of thawing. For normal serum or plasma samples, 2-fold dilution into Diluent 2 is recommended, but generally not required. Serum or plasma with higher-than-normal levels of MMP may require a higher dilution ratio. EDTA and citrate as anticoagulants are not recommended due to their chelating properties, since activity of MMPs requires zinc and calcium.

Tissue Culture

Tissue culture supernatant samples may not require dilution prior to being used in the MSD Human MMP 2-Plex 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 Blend is provided at 50X stock solution. 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 Detection Antibody Blend into 2.94 mL of Diluent 3.

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 2:** Dispense 25 μL of Diluent 2 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 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 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

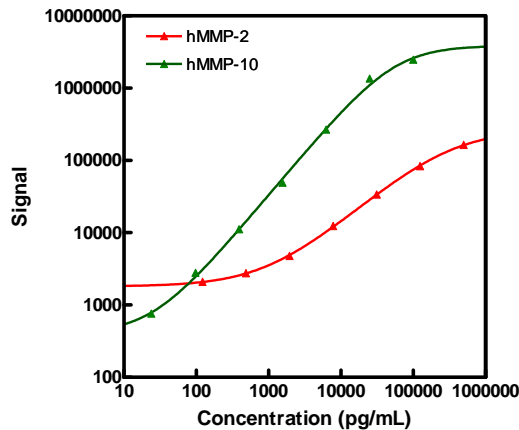
analysis of results

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 curves are 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.



MMP-2		
Conc. (pg/mL)	Average Signal	%CV
0	1898	4.9
122	2090	4.9
488	2749	5.4
1953	4778	3.7
7813	12369	3.6
31250	33802	2.3
125000	83397	4.6
500000	163908	2.7

MMP-10		
Conc. (pg/mL)	Average Signal	%CV
0	44	15.2
24	764	3.7
98	2798	4.8
391	11190	2.6
1563	49469	3.5
6250	266084	4.1
25000	1350749	3.2
100000	2467232	0.8

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 values below represent the average LLOD over multiple kit lots.

	MMP-2	MMP-10
LLOD (pg/mL)	120	14

XI Specificity

specificity

To determine the specificity of the assays, MMP Calibrators were assayed individually in Diluent 2 with MMP 2-plex plates. The MMP-10 Calibrator displayed 3% crossreactivity with the MMP-2 spot. Recombinant human MMP-1, MMP-3, MMP-9, MMP-7, TIMP-1 and TIMP-2 proteins were also tested. Less than 0.1% crossreactivity was observed for MMP- 2 and 10 assays with MMP-1, MMP-3, MMP-9, MMP-7, TIMP-1 and TIMP-2 proteins.

XII Assay Components

Assay components

The human MMP-2 and MMP-10 capture and detection antibodies used in this assay are listed below.

Analyte	Source species	
	MSD Capture Antibody	MSD Detection Antibody
MMP-2	Goat Polyclonal	Goat Polyclonal
MMP-10	Mouse Monoclonal	Goat Polyclonal

Summary Protocol

MSD 96-well MULTI-SPOT Human MMP 2-Plex Assay: Ultra-Sensitive Kit

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

Step 1: Sample and Reagent Preparation

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

If necessary, samples should be diluted in Diluent 2.

Prepare calibrator solutions and standard curve.

Use the 10X Calibrator stock to prepare an 8-point standard curve by diluting in Diluent 2.

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

Prepare Detection Antibody Solution by diluting Detection Antibody Blend to 1X in a final volume of 3.0 mL Diluent 3 per plate.

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

SERUM OR PLASMA SAMPLES

Step 2: Add Diluent 2

Dispense 25 μ L/well Diluent 2.

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

Step 3: Add Sample or Calibrator

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 2X Read Buffer T.

Analyze plate on SECTOR Imager instrument.

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