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Enabling Legendary Discovery™

LEGEND MAX™

ELISA Kit with Pre-coated Plate

Human MMP-9

Cat. No. 444907

ELISA Kit for Accurate Quantitation of Human MMP-9 from Serum, Plasma, Cell Culture Supernatant, Urine, and Saliva

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biolegend.com

It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.

Table of Contents Page

Introduction:

Matrix metalloproteinase 9 (MMP-9), also known as gelatinase B or 92 kD type IV collagenase, belongs to the family of MMPs, which are zinc-dependent enzymes whose primary function is to degrade extracellular matrix proteins. MMP-9 is generally secreted as an inactive 92 kD pro-form by many cell types, notably neutrophils, monocytes, macrophages, endothelial cells, platelets, and tumor cells. A cysteine residue in the pro-domain coordinates with zinc in the catalytic domain to confer latency. When the pro-domain is cleaved off by other proteases, such as MMP-3, the enzyme is activated and forms its typical 82 kD mature form. An atypical 65 kD mature form also exists that lacks the hemopexin domain.

MMP-9 is the only MMP that can form homodimers, and both pro- and mature homodimer forms can exist. MMP-9 can also interact and bind to a multitude of other proteins. For example, tissue inhibitor of metalloproteinase 1 (TIMP-1) is its natural inhibitor. Other binding partners include CD44 and neutrophil gelatinase-associated lipocalin (NGAL).

MMP-9 is most closely related to MMP-2, with which it shares only 46% amino acid sequence identity overall. Across species, human MMP-9 shares 82-99%, 75%, and 72% amino acid sequence identity with non-human primate, rat, and mouse MMP-9, respectively.

MMP-9 plays important roles in inflammation, wound healing, tissue remodeling, cell migration, and tumor growth and metastasis. It is a potential biomarker for many diseases and conditions, often with elevated levels seen in various cancers, inflammatory conditions, allergies, autoimmune diseases, and myocardial infarction.

The LEGEND MAX™ Human MMP-9 Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kit includes a 96-well strip plate that is pre-coated with a mouse monoclonal anti-human MMP-9 antibody. The Detection Antibody is a biotinylated mouse monoclonal anti-human MMP-9 antibody. This kit is specifically designed to accurately measure MMP-9, including monomeric and dimeric pro-MMP-9, MMP-9/NGAL complex, and MMP-9/NGAL/TIMP-1 complex. This kit has been analytically validated to assess cell culture supernatant, urine, saliva, serum, and plasma samples with ready-to-use reagents.

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Materials Provided:

Description	Quantity	Volume (per bottle)	Part #
Anti-human MMP-9 Pre-coated 96-well Strip Microplate	1 plate		76280
Human MMP-9 Detection Antibody	1 bottle	12 mL	76281
Human MMP-9 Standard	1 vial	lyophilized	76283
Avidin-HRP	1 bottle	12 mL	77897
Assay Buffer A	2 bottles	25 mL	78232
Wash Buffer (20X)	1 bottle	50 mL	78233
Substrate Solution D	1 bottle	12 mL	76337
Stop Solution	1 bottle	12 mL	79133
Plate Sealers	4 sheets		78101

Materials to be Provided by the End-User:

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Plate Shaker
- Polypropylene vials

Storage Information:

Store unopened kit components between 2°C and 8°C. Do not use this kit beyond its expiration date.

Opened or Reconstituted Components	
Microplate Strips	If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store between 2°C and 8°C for up to one month.
Standard	The remaining reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.
Detection Antibody	Store opened reagents between 2°C and 8°C and use within one month.
Avidin-HRP	
Assay Buffer A	
Wash Buffer (20X)	
Substrate Solution D	
Stop Solution	

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend's website for details (www.biolegend.com/msds).
2. Substrate Solution D is harmful if inhaled or ingested. Avoid skin, eye and clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
4. Stop Solution contains strong acid and is corrosive. *Wear eye, hand, and face protection.*
5. Before disposing of the plate, rinse it with an excess amount of tap water.

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Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantitation.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 20 minutes at 1,000 x *g*. Remove serum layer and assay immediately or store serum samples at < -70°C. Avoid repeated freeze-thaw cycles.

Plasma: Collect blood samples in heparin-containing tubes, and ensure a clean stick with no hemolysis. Centrifuge immediately for 20 minutes at 1,000 x *g*. Carefully remove supernatant and assay immediately or store at < -70°C. Avoid repeated freeze-thaw cycles. ***EDTA and citrate are not recommended for plasma preparation due to reduced detectability of MMP-9 in the plasma.***

Cell Culture Supernatant: If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at < -70°C. Avoid repeated freeze-thaw cycles.

Urine: Collect first-morning, mid-stream urine samples in a sterile container. To remove heavy sediment, centrifuge for 10 minutes at 1,000 x g. Remove supernatant and assay immediately or store at < -70°C. Avoid repeated freeze-thaw cycles.

Saliva: If utilizing a saliva collection device, ensure that it will not bind to proteins. To remove heavy sediment, centrifuge for 10 minutes at 1,000 x g. Remove supernatant and assay immediately or store at < -70°C. Avoid repeated freeze-thaw cycles.
NOTE: Because there are very high levels of MMP-9 in saliva, care must be taken to avoid contamination of reagents and samples from end-user's saliva. Consider utilizing a face mask or shield.

Reagent and Sample Preparation:

Note: All reagents should be diluted immediately prior to use.

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
2. Reconstitute the lyophilized Human MMP-9 Standard by adding the volume of Assay Buffer A to make a 100 ng/mL standard stock solution (Refer to LEGEND MAX™ Kit Lot-Specific Certificate of Analysis/LEGEND MAX™ Kit Protocol). Allow the reconstituted standard to sit at room temperature for 15 minutes, then briefly vortex to mix completely.
3. For measuring serum samples, a 200-fold dilution is recommended using Assay Buffer A. For example, dilute 3 µL of sample in 597 µL of Assay Buffer A.
4. For plasma samples, a 100-fold dilution is recommended using Assay Buffer A. For example, dilute 3 µL of sample in 297 µL of Assay Buffer A.
5. For cell culture supernatant samples, MMP-9 concentration varies, so multiple dilutions should be performed. Dilution is recommended using matched cell culture media or Assay Buffer A.
6. For urine samples, in general, no sample dilution is required. If dilutions are necessary, samples should be diluted with Assay Buffer A.
7. For saliva samples, an 80-fold dilution is recommended using Assay Buffer A. For example, dilute 5 µL in 395 µL of Assay Buffer A.

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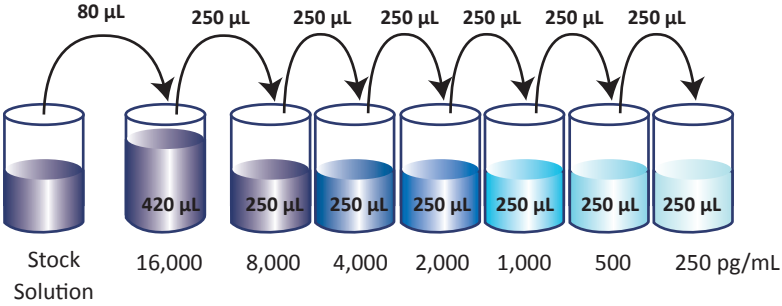
Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.
3. Prepare 500 µL of the 16,000 pg/mL top standard by diluting 80 µL of the standard stock solution in 420 µL of Assay Buffer A. Perform six two-fold serial dilutions of the 16,000 pg/mL top standard in separate tubes using Assay Buffer A as the diluent. Thus, the human MMP-9 standard concentrations in the tubes are 16,000 pg/mL, 8,000 pg/mL, 4,000 pg/mL, 2,000 pg/mL, 1,000 pg/mL, 500 pg/mL, 250 pg/mL, respectively. Assay Buffer A serves as the zero standard (0 pg/mL).



4. Add 50 µL Assay Buffer A to each well that will contain either standards or samples.
5. Add 50 µL of standard dilutions or properly diluted samples to the appropriate wells.
6. Seal the plate with a Plate Sealer included in the kit and incubate the plate at room temperature for 2 hours while shaking at 200 rpm.
7. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer. Wash the plate with at least 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping plate upside down on absorbent paper. All subsequent washes should be performed similarly.
8. Add 100 µL of Human MMP-9 Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.

9. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 7.

10. Add 100 µL of Avidin-HRP solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.

11. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 7. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.

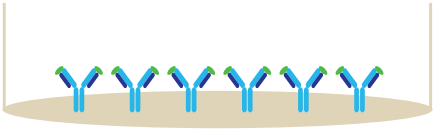
12. Add 100 µL of Substrate Solution D to each well and incubate at room temperature for 15 minutes in the dark. Wells containing human MMP-9 should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.

13. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.

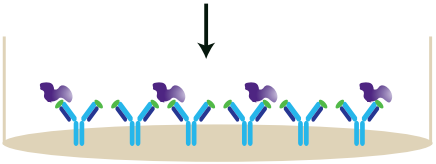
14. Read absorbance at 450 nm immediately. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

Assay Procedure Summary

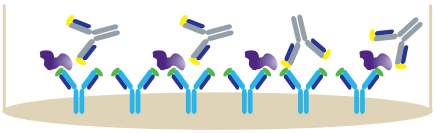
1. Add 50 µL Assay Buffer A



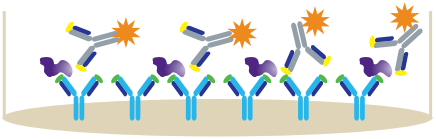
2. Add 50 µL diluted standards or samples
   Incubate 2 hrs, RT, shaking



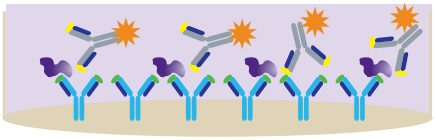
3. Wash 4 times
   Add 100 µL Detection Antibody solution
   Incubate 1 hr, RT, shaking



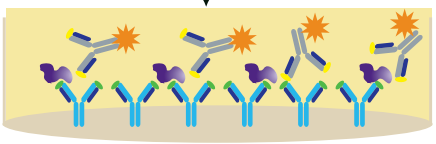
4. Wash 4 times
   Add 100 µL Avidin-HRP solution
   Incubate 30 mins, RT, shaking



5. Wash 5 times
   Add 100 µL Substrate Solution D
   Incubate 15 mins, RT, in the dark



6. Add 100 µL Stop Solution



7. Read absorbance at 450 nm and 570 nm

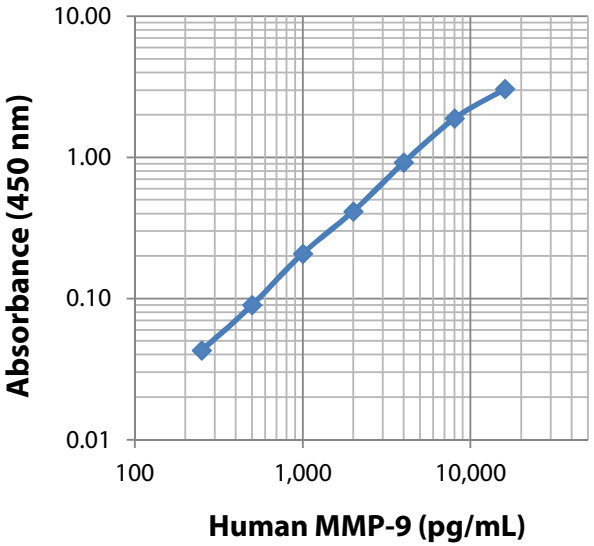
Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with protein concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown protein concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the protein concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

Typical Data:

This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



Performance Characteristics:

Specificity: No cross-reactivity was observed when this kit was used to analyze the following recombinant proteins, each at the specified concentration.

Human	100 ng/mL: MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-10, MMP-11, MMP-12, MMP-13, MMP-14, MMP-15, MMP-16, TIMP-1, TIMP-2, TIMP-3, TIMP-4, NGAL 50 ng/mL: IFN-γ, TNF-β, CCL11, CCL22, MRP8
Mouse	100 ng/mL: MMP-2, MMP-3, MMP-9, TIMP-1 50 ng/mL: NGAL

Sensitivity: The minimum detectable concentration is 46.9 pg/mL ± 15.0 pg/mL (mean ± 2SD; n=9).

Linearity: Human samples were diluted with Assay Buffer A to produce samples with values within the dynamic range, and then assayed with the kit to determine the dilutional linearity up to 8-fold dilutions. For urine, samples were spiked with recombinant human MMP-9/NGAL/TIMP-1 prior to dilutions.

Sample Type	N	% Linearity
Serum	10	109
Plasma	5	90
Cell culture supernatant	4	105
Urine	4	112
Saliva	5	115

Recovery: Recombinant human MMP-9 was spiked at the listed concentrations into the following human samples, with the exception of urine, into which recombinant human MMP-9/NGAL/TIMP-1 complex was spiked. **Samples were diluted prior to spiking.*

Sample Type	Spike (pg/mL)	N	% Recovery
Cell culture supernatant*	5,000, 1,250, 313	4	112
Urine	8,000, 2,000, 500	4	95
Saliva*	8,000, 2,000, 500	5	118

Intra-Assay Precision: Two samples with different concentrations of human serum were tested with 16 replicates in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	8,509	2,018
Standard Deviation	266	38
% CV	3.1	1.9

Inter-Assay Precision: Two different concentrations of human serum were assayed in four independent assays by four different users.

Concentration	Sample 1	Sample 2
Number of Assays	4	4
Mean Concentration (pg/mL)	7,541	1,720
Standard Deviation	672	229
% CV	8.9	13.3

Biological Samples

Serum, Plasma, Urine, and Saliva: Matched serum and plasma samples from 15 donors, urine samples from 4 donors, and saliva samples from 5 donors were tested in one assay.

Sample Type	N	% Detectable	Min. (ng/mL)	Max. (ng/mL)	Median (ng/mL)
Serum	15	100	655	3,122	1,042
Plasma	15	100	30	463	222
Urine	4	25	ND*	0.68	ND*
Saliva	5	100	50	458	164

**ND = Not Detectable*

Cell Culture Supernatant: Human THP-1 cells and two PBMC cultures from the same source were established in RPMI/10% FBS at a concentration of $1x10^6$ cells/mL. One PBMC culture was stimulated with LPS (100 ng/mL) immediately after the culture was seeded, and the other culture was not stimulated. Human NB4 cells were established in DMEM/10% FBS at a concentration of $1x10^6$ cells/mL. Each culture was grown for a period of 4 days. The supernatant from each of the cultures was collected on day 4, and the THP-1, NB4, and PBMC supernatants were measured to have the concentrations of MMP-9 listed below.

Culture	Treatment	Time Point	Concentration (ng/mL)
THP-1	Unstimulated	Day 4	30.7
NB4	Unstimulated	Day 4	17.5
PBMC	Unstimulated	Day 4	50.4
	LPS	Day 4	65.1

Troubleshooting Guide:

Problem	Probable Cause	Solution
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents.
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN3)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

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Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

ELISA Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Notes

LEGEND MAX™ Kits are manufactured by **BioLegend Inc.**
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