BioLegend®

Enabling Legendary Discovery™

LEGEND MAX™

ELISA Kit with Pre-coated Plates

Human Total MMP-2

Cat. No.	444607	1 Plate
	444608	5 Plates

ELISA Kit for Accurate Quantitation of Human Total MMP-2 from Cell Culture Supernatant, Serum, and Heparin Plasma

BioLegend, Inc.
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.

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Introduction:

Matrix metalloproteinase 2 (MMP-2), also known as gelatinase A or 72kD type IV collagenase, is a member of a broad family of MMPs, which are zinc-dependent enzymes that break down extracellular matrix proteins as part of their primary functions. MMP-2 is secreted in its 72 kD latent pro form from many cell types including neutrophils, monocytes, macrophages, endothelial cells, platelets, and tumor cells. It exists as a monomer and can form a homodimer that is known to regulate pro MMP-2 activation. When its pro-domain is cleaved by proteases such as MMP-14 and thrombin, it becomes the active 62 kD mature form. Activated MMP-2 degrades collagenase of the basement membrane and gelatin as well as several chemokines and cytokines. MMP-2 can also bind to other proteins, such as the tissue inhibitors of metalloproteinase (TIMPs). Specifically, TIMP-2 is able to bind to MMP-2 and MMP-14 on the cell surface to form a complex that regulates MMP-2 activation.

MMP-2 is closely related to MMP-9, although it only shares 46% amino acid sequence identity. Human MMP-2 shares significant (>95%) amino acid sequence identity with MMP-2 from other species, including non-human primates, equine, porcine, mouse, bovine, and rat.

MMP-2 plays important roles in vasculature remodeling, angiogenesis, tissue repair, tumor invasion, inflammation, and atherosclerotic plaque rupture. MMP-2 is a potential biomarker for many diseases and conditions, as it is involved in tumor metastasis, inflammatory conditions, allergies, autoimmune diseases, and myocardial infarction.

The LEGEND MAX™ Human Total MMP-2 Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kit includes a 96-well strip plate that is pre-coated with a monoclonal mouse anti-human MMP-2 antibody. The Detection Antibody is a biotinylated polyclonal rabbit anti-human MMP-2 antibody. This kit is specifically designed to accurately measure MMP-2, including pro MMP-2, mature MMP-2, and mature MMP-2/TIMP-2 complex. This kit has been analytically validated to assess MMP-2 levels in cell culture supernatant, serum, and heparin plasma samples with ready-to-use reagents.

Tel: 858-768-5800

Materials Provided:

Description	Quantity (1 plate)	Quantity (5 plates)	Volume (per bottle)	Part #
Anti-human Total MMP-2 Pre-coated 96-well Strip Microplate	1 plate	5 plates		76960
Human Total MMP-2 Detection Antibody	1 bottle	5 bottles	12 mL	76961
Human Total MMP-2 Standard	1 vial	5 vials	lyophilized	76963
Avidin-HRP	1 bottle	5 bottles	12 mL	77897
Assay Buffer B	1 bottle	5 bottles	25 mL	79128
Wash Buffer (20X)	1 bottle	5 bottles	50 mL	78233
Substrate Solution F	1 bottle	5 bottles	12 mL	76335
Stop Solution	1 bottle	5 bottles	12 mL	79133
Plate Sealers	4 sheets	20 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.

<table>
<tr><th colspan="2">Opened or Reconstituted Components</th></tr>
<tr><td>Microplate Strips</td><td>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.</td></tr>
<tr><td>Standard</td><td>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.</td></tr>
<tr><td>Detection Antibody</td><td rowspan="6">Store opened reagents between 2°C and 8°C and use within one month.</td></tr>
<tr><td>Avidin-HRP</td></tr>
<tr><td>Assay Buffer B</td></tr>
<tr><td>Wash Buffer (20X)</td></tr>
<tr><td>Substrate Solution F</td></tr>
<tr><td>Stop Solution</td></tr>
</table>

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 F 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.

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.

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-2 in the plasma.***

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 Total MMP-2 Standard by adding the volume of Assay Buffer B 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 or plasma samples, a 100-fold dilution is recommended using Assay Buffer B. For example, dilute 2 µL of sample in 198 µL of Assay Buffer B. For cell culture supernatant samples, in general, at least a 2-fold dilution is recommended using the matched cell culture media. For example, dilute 100 µL of sample in 100 µL of matched cell culture media.

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.

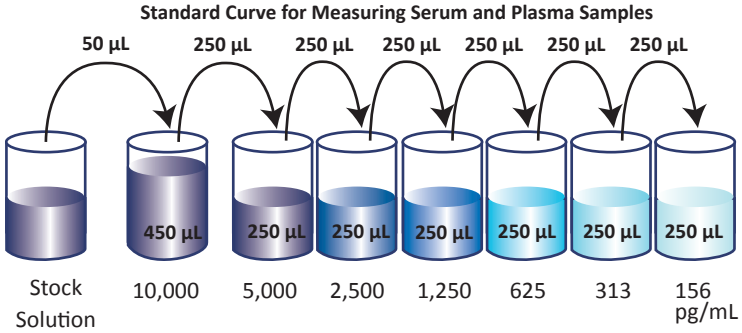
3. For measuring serum and plasma samples:

   a) Prepare 500 µL of the 10,000 pg/mL top standard by diluting 50 µL of the standard stock solution in 450 µL of Assay Buffer B.

   b) Perform six two-fold serial dilutions of the 10,000 pg/mL top standard in separate tubes using Assay Buffer B as the diluent. Thus, the human Total MMP-2 standard concentrations in the tubes are 10,000 pg/mL, 5,000 pg/mL, 2,500 pg/mL, 1,250 pg/mL, 625 pg/mL, 313 pg/mL, 156 pg/mL, respectively. Assay Buffer B serves as the zero standard (0 pg/mL).

   c) Add 50 µL Assay Buffer B to each well that will contain either standard dilutions or samples.

   d) Add 50 µL of standard dilutions or samples to the appropriate wells.



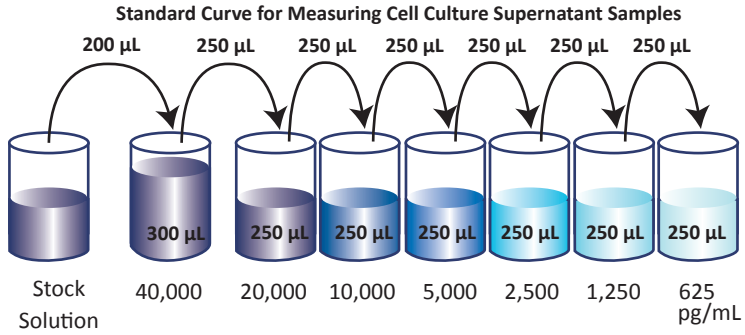
For testing cell culture supernatant samples:

a) Prepare 500 µL of the 40,000 pg/mL top standard by diluting 200 µL of the standard stock solution in 300 µL of Assay Buffer B.

b) Perform six two-fold serial dilutions of the 40,000 pg/mL top standard in separate tubes using Assay Buffer B as the diluent. Thus, the human Total MMP-2 standard concentrations in the tubes are 40,000 pg/mL, 20,000 pg/mL, 10,000 pg/mL, 5,000 pg/mL, 2,500 pg/mL, 1,250 pg/mL, 625 pg/mL, respectively. Assay Buffer B serves as the zero standard (0 pg/mL).

c) Add 50 µL matched cell culture media to each well that will contain standard dilutions. Add 50 uL Assay Buffer B to each well that will contain samples.

d) Add 50 µL of standard dilutions to the wells containing matched cell culture media. Add 50 µL of samples to the wells containing Assay Buffer B.



4. 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.

5. 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.

6. Add 100 µL of Human Total MMP-2 Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.

7. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 5.

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

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

10. Add 100 µL of Substrate Solution F to each well and incubate at room temperature for 15 minutes in the dark. Wells containing human Total MMP-2 should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.

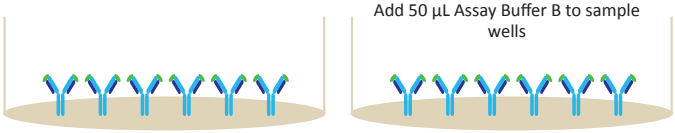
11. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.

12. 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

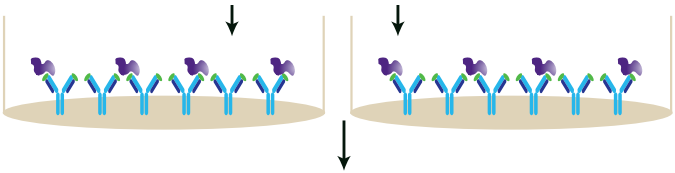
1. **For measuring serum or plasma:**
Add 50 µL Assay Buffer B to standard and sample wells

For measuring cell culture supernatant:
Add 50 µL matched cell culture media to standard wells
Add 50 µL Assay Buffer B to sample wells

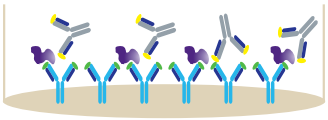


2. Add 50 µL diluted standards to standard wells
Add 50 µL diluted samples to sample wells
Incubate 2 hr, RT, shaking

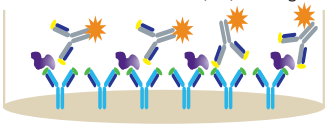
Add 50 µL diluted standards to standard wells
Add 50 µL diluted samples to sample wells
Incubate 2 hr, RT, shaking



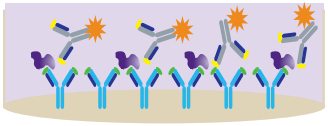
3. Wash 4 times
Add 100 µL Detection Antibody solution
Incubate 1 hr, RT, shaking



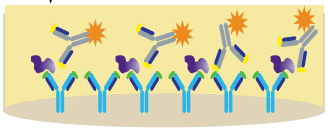
4. Wash 4 times
Add 100 µL Avidin-HRP solution
Incubate 30 min, RT, shaking



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



6. Add 100 µL Stop Solution



7. Read absorbance at 450 nm and 570 nm

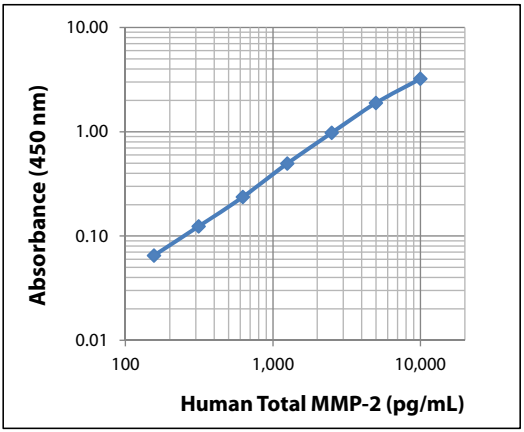
Calculation of Results:

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 for measuring serum and heparin plasma 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 100 ng/mL except where the concentration is specified.

Human	MMP-1, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-11, MMP-12, MMP-13, MMP-14, MMP-15, MMP-16, TIMP-1, TIMP-2, CCL1 (50 ng/mL), IFN-γ (50 ng/mL), NGAL
Mouse	MMP-3, MMP-9, NGAL

This kit has ~1.16% cross-reactivity with mouse MMP-2. This kit accurately detects human pro MMP-2, mature MMP-2, mature MMP-2/TIMP-2 complex.

Sensitivity: The minimum detectable concentration is 43.8 pg/mL ± 15.9 pg/mL (n=12) for measuring serum and heparin plasma and 193.5 ± 138.5 pg/mL (n=3) for measuring cell culture supernatant (mean ± 2 SDs).

Linearity: Human samples were diluted with Assay Buffer B or matched cell culture media 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.

Sample Type	N	% Linearity
Serum	5	117
Heparin Plasma	5	95
Cell Culture Supernatant	4	82

Recovery: Recombinant human Total MMP-2, at concentrations of 20,000, 5,000, and 1,250 pg/mL, was spiked into cell culture samples.

Sample Type	N	% Recovery
Cell Culture Supernatant*	4	116

**Samples were diluted prior to spiking.*

Intra-Assay Precision: Two samples of human serum diluted differently in Assay Buffer B were tested with 16 replicates in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	4157.8	688.4
Standard Deviation	213.0	65.3
% CV	5.1	9.5

Inter-Assay Precision: Two samples of human serum diluted differently in Assay Buffer B were assayed in four independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	4	4
Mean Concentration (pg/mL)	2702.2	607.6
Standard Deviation	183.8	62.3
% CV	6.8	10.3

Biological Samples: Serum and plasma samples from 19 donors were tested in one assay.

Sample Type	N	% Detectable	Min. (ng/mL)	Max. (ng/mL)	Median (ng/mL)
Serum	19	100	26.3	592.1	235.7
Heparin Plasma	19	100	98.3	502.6	281.0

Cell Culture Supernatant - Human THP-1, NB4, HepG2, U87-MG, and DAUDI cells were established in RPMI/10% FBS at a concentration of $1x10^6$/mL and incubated for a period of 4 days. Supernatant from the culture was collected on day 4, and the concentrations of MMP-2 in each supernatant are listed below.

Cell Type	Time	Concentration (ng/mL)
THP-1	Day 4	9.5
NB4	Day 4	8.1
HepG2	Day 4	7.5
U87-MG	Day 4	410.7
DAUDI	Day 4	ND*

**ND = Not Detectable*

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.

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

Tel: 858-768-5800

Notes

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