Human MMP-2 ELISA Kit

Catalog Number KHC3081 (96 tests), KHC3082 (2 × 96 tests)

Pub. No. MAN0009796 **Rev.** 2.0

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ Human MMP-2 ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of human MMP-2 in human serum, plasma, buffered solution, or cell culture medium.

Matrix metallopeptidase-2 (MMP-2), is also known as gelatinase A, 72 kDa gelatinase, and 72 kDa type IV collagenase. Human, mouse and rat MMP-2 share about 95% amino acid sequence identity. MMP-2 has five distinct domains: a propeptide domain which is cleaved upon activation, a gelatin-binding domain consisting of three contiguous fibronectin type II units, a catalytic domain containing the zinc binding motif, a proline-rich linker region, and a carboxyl terminal hemopexin-like domain. MMP-2 is primarily expressed in mesenchymal cells, such as fibroblasts, during development and tissue regeneration. MMP-2 is secreted from cells as an inactive zymogen. The N-terminal propeptide domain contains the cysteine switch motif that maintains MMP-2 in a latent state. MMP-2 is activated by removal of pro-peptide domain to yield mature active form. This proteolytic cleavage is mediated by membrane-type MMPs or serine proteases. TIMPs inhibit active MMP-2 through tight, but non-covalent binding of their N-terminal domains to the catalytic domain of MMP-2.

Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No. KHC3081 (96 tests)
Hu MMP-2 Standard, lyophilized; contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume	2 vials
Standard Diluent Buffer, contains 0.1% sodium azide; red dye [1]	25 mL
Incubation Buffer	12 mL
Antibody Coated Wells, 96-well strip-well plate	1 plate
Hu MMP-2 Biotin Conjugate; contains 0.1% sodium azide	11 mL
Streptavidin-HRP (100X), contains 3.3 mM thymol	0.125 mL
Streptavidin HRP Diluent, contains 3.3 mM thymol	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Plate Covers, adhesive strips	3

[1] In order to help our customers avoid any mistakes in pipetting the ELISAs, we provide colored Standard Diluent Buffer, Detection Antibody and HRP Diluent to help monitor the addition of solutions to the reaction wells. This does not in any way interfere with the test results.

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Pre-dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

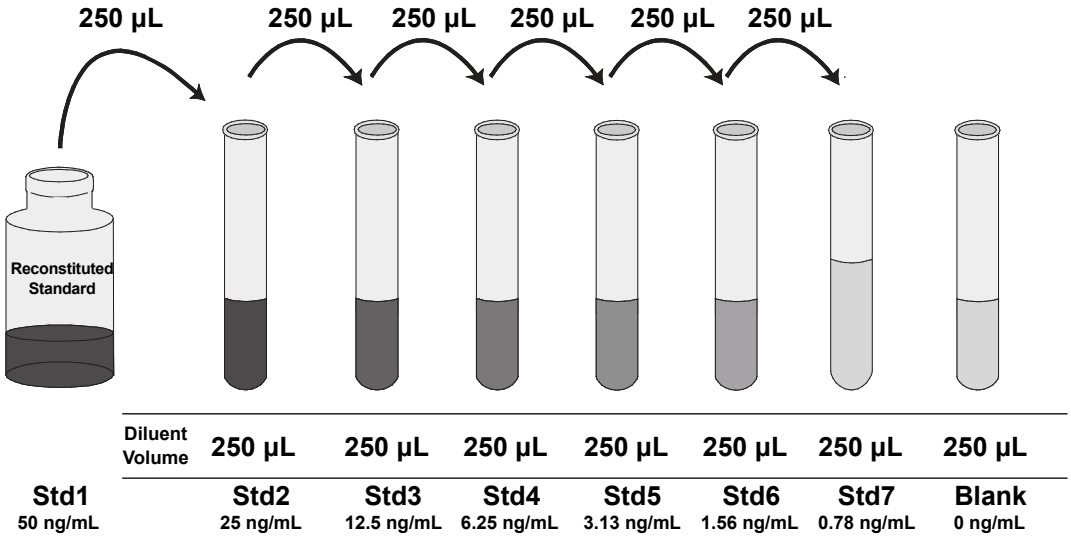
- Perform sample dilutions with Standard Diluent Buffer.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Note: The Human MMP-2 Standard is prepared from a highly purified recombinant protein.

1. Reconstitute Human MMP-2 Standard to 50 ng/mL with Standard Dilution Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 50 ng/mL human MMP-2. Use the standard within 1 hour of reconstitution.
2. Add 250 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 25, 12.5, 6.25, 3.13, 1.56, 0.78, and 0 ng/mL human MMP-2.
3. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
4. Discard any remaining reconstituted standard. Return the Standard Diluent Buffer to the refrigerator.



Prepare 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

The Streptavidin-HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 4 hours)

IMPORTANT! Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 Bind antigen

a. Add 50 µL of the Incubation Buffer to all wells except the chromogen blanks.
b. Add 50 µL of standards, controls, or samples (see "Pre-dilute samples" on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
c. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate for 2 hours at room temperature.
d. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Biotin Conjugate

a. Add 100 µL Hu MMP-2 Biotin Conjugate solution into each well except the chromogen blanks.
b. Cover the plate with plate cover and incubate for 1 hour at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

3 Add Streptavidin-HRP

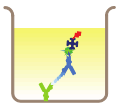
a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

4 Add Stabilized Chromogen

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

5 Add Stop Solution



Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve example

The following data were obtained for the various standards over the range of 0 to 50 ng/mL MMP-2.

Standard Human MMP-2 (ng/mL)	Optical Density (450 nm)
50	3.05
25	1.91
12.5	1.09
6.25	0.60
3.13	0.35
1.56	0.22
0.78	0.15
0	0.10

Inter-assay precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	23.5	6.2	1.6
Standard Deviation	1.2	0.4	0.1
% Coefficient of Variation	5.3	6.0	5.5

Intra-assay precision

Samples of known Hu MMP-2 concentration were assayed in replicates of 16 to determine precision within an assay.

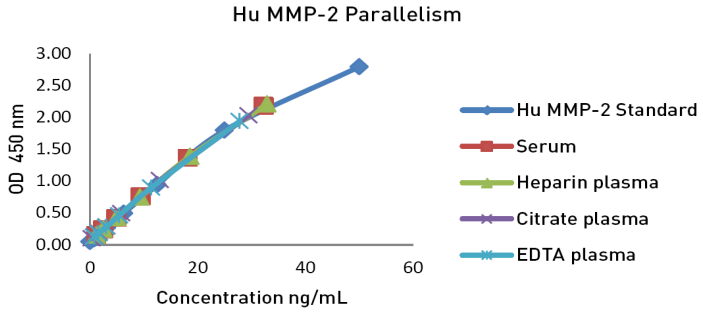
Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	24	6.4	1.6
Standard Deviation	0.8	0.2	0.1
% Coefficient of Variation	3.2	3.3	3.5

Linearity of dilution

Human serum, citrate plasma, heparin plasma, and EDTA plasma spiked with natural Hu MMP-2 were serially diluted in Standard Diluent Buffer over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded average correlation coefficients of 0.99 for serum, citrate plasma, heparin plasma, and EDTA plasma.

Parallelism

Human serum, citrate plasma, heparin plasma, EDTA plasma, and cell culture medium spiked with natural Hu MMP-2 were serially diluted in Standard Diluent Buffer over the range of the assay. The standard accurately reflects the natural MMP-2 content in the samples.



Recovery

The recoveries of Hu MMP-2 added to human serum, citrate plasma, heparin plasma, EDTA plasma, and tissue culture media containing 10% fetal bovine serum (FBS) were measured with the Hu MMP-2 ELISA Kit.

Sample	Average % Recovery
Serum	92
Citrate plasma	87
Heparin plasma	93
EDTA plasma	84
DMEM with 10% FBS	101

Sensitivity

The analytical sensitivity of Hu MMP-2 is <0.15 ng/mL. This was determined by adding two standard deviations to the mean optical density (OD) obtained when the zero standard was assayed 40 times, and calculating the corresponding concentration.

Specificity

Buffered solutions of a panel of substances ranging in concentrations from 1.3 to 144 ng/mL were assayed with the Hu MMP-2 Kit and found to have no cross-reactivity: **human** Eotaxin, GM-CSF, IFN-α, IFN-γ, IL-1β, IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 p40/p70, IL-13, IL-15, IL-16, IL-17, IP-10, MCP-1, MIG, MIP-1α, MIP-1β, MMP-3, MMP-13, RANTES, TIMP-1, TIMP-2, TIMP-4, PAI-1, C-peptide, BAFF, Leptin, Lipocalin-2, Insulin, SAA, Resistin, ICAM-1, VCAM-1, E-Selectin, P-Selectin, PECAM-1, TNF-α, HGF, G-CSF, VEGF, FGF-basic, and EGF; **rat** GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12 and TNF-α; **mouse** IL-1α, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-17, IP-10, MIP-1α, KC, MCP-1, TNF-α, and VEGF.

Cross-reactivity

Recombinant human MMP-9 protein demonstrated 4% cross-reactivity with this kit.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Manufacturer's address: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

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