

Human MMP9 ELISA Kit

Sandwich ELISA

Catalog EL-213-13131609

INTRODUCTION

MMP9 encodes gelatinase B, a secreted zinc-dependent enzyme released by neutrophils, macrophages and several other cell types. Once activated by cleavage of its inactive proform, the enzyme breaks down type IV and V collagen along with fibronectin, allowing it to remodel the extracellular matrix. This remodeling activity supports normal processes such as embryonic development, wound repair and the mobilization of bone-marrow progenitor cells, while excessive or dysregulated activity contributes to inflammatory joint disease, tumor invasion and metastatic spread. Given its dual role in tissue homeostasis and pathological remodeling, MMP9 is widely measured as a marker of inflammation and matrix turnover.

PRINCIPLE OF THE ASSAY

This kit utilizes a sandwich enzyme immunoassay format. The microtiter plate is pre-coated with a capture antibody specific to Human MMP9; a separate set of standard plate wells pre-coated by protein-related techniques is provided for the standard curve. Standards or samples are dispensed into the appropriate wells, followed by addition of an HRP-conjugated detection antibody that also targets Human MMP9. Upon addition of TMB substrate solution, a colorimetric signal develops exclusively in wells containing both Human MMP9 and the HRP-conjugated antibody. The enzymatic reaction is terminated by addition of sulfuric acid stop solution, and absorbance is measured at 450 nm (± 10 nm). Sample concentrations are then determined by comparing the optical density of each sample against the generated standard curve.

MATERIALS AND STORAGE

Store kit components at -20°C unless specified otherwise. DO NOT USE past kit expiration date. Some vials contain a small amount of reagents. Spin tubes on pulse setting prior to opening.

Each kit includes:	Units
Pre-Coated Microplate	12 strips x 8 wells
Standard Microplate	2 strips x 8 wells
HRP Conjugate Antibody (100×)	70 µL
Standard/Sample Diluent Buffer	24 mL
HRP Conjugate Diluent	10 mL
Wash Buffer (25×)	24 mL
TMB Substrate Solution	12 mL
Stop Reagent	7 mL
Plate Covers	2 pieces
Instruction Manual	1
<i>Do not mix or substitute reagents with those from other lots.</i>	

MATERIALS AND INSTRUMENTS REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 5-1000µL
- Multi-channel pipette calibrated to deliver 50-200µL
- Plate shaker
- Disposable tips
- Vortex-Mixer
- Distilled or de-ionized water
- Microplate reader capable of reading 450nm with background subtraction at 620nm

SAFETY PRECAUTIONS

- The test protocol must be followed strictly.
- All reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient

sera or serum based products.

- The kit reagents contain antimicrobial agents, acid and 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
- Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
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- Only trained laboratory personnel should execute this test.

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PREPARATION OF REAGENTS

Prepare only the appropriate amount of required reagent on the day of use. Store all reagents as per instructions stated on the label.

- 1. Wash Buffer (1X) Preparation: Dilute the 25X wash buffer concentrate 1:25 with double-distilled Water.**
- 2. Detection Reagent (1X) Preparation: Dilute the HRP-conjugated detection antibody concentrate 100-fold with HRP Conjugate Diluent, immediately before use.**
- 3. Preparation of Calibrators: Prepare calibrators by serial dilution from a 80 down to 1.25 (plus a 0 blank), per the source manual.**

Sol'n ID	Source	Source Vol (μL)	Cal. Diluent (μL)	Final Vol (μL)	Final Conc. (ng/mL)
Std 1	Stock Standard	500	500	1000	80
Std 2	Std 1	500	500	1000	40
Std 3	Std 2	500	500	1000	20
Std 4	Std 3	500	500	1000	10
Std 5	Std 4	500	500	1000	5
Std 6	Std 5	500	500	1000	2.5
Std 7	Std 6	500	500	1000	1.25
Blank	Std 7	500	500	1000	0

SPECIMEN STORAGE

This kit is compatible with EDTA-plasma, heparin-plasma and serum samples. Samples can be stored at or below -20°C for up to 1 year.

ASSAY PROCEDURE

1. After the kit is equilibrated at room temperature, add 50μL Standard/Sample Diluent Buffer to each Standard well , and add 50 μL sample to the sample well, Immediately add 50 μL 1× HRP Conjugate Antibody Working Solution to each well, Incubate at 37°C on Microplate . oscillator for 60 min

2. Discard the liquid in the plate, add 200 μL 1× Wash Buffer to each well, and wash the plate 5 times. After pat it dry against clean absorbent paper, add 90 μL TMB Substrate Solution to each well, incubate at 37°C for 20 minutes in the dark.

3. Add 50 μL Stop Solution to each well, shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm immediately, calculation of the results.

CALCULATIONS AND RESULTS

- Construct a standard curve by plotting the absorbance obtained from each standard against concentration. Use a 4 or 5 parameter curve fit. Alternatively a log-log curve fit may be used.
- The concentration of the unknowns can be read directly from this standard curve using the absorbance value for each sample.
- Any sample undiluted or diluted still reading greater than the highest standard should be diluted appropriately and retested. If the samples have been diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

PERFORMANCE CHARACTERISTICS

Precision: Intra-assay CV < 8%. Inter-assay CV < 10%.

Detection Limit: 1.25 ng/mL

Recovery: Serum (n = 5): 90-105% (avg 97%); EDTA plasma (n = 5): 80-97% (avg 88%)

88%); Heparin plasma (n = 5): 78-92% (avg 85%)

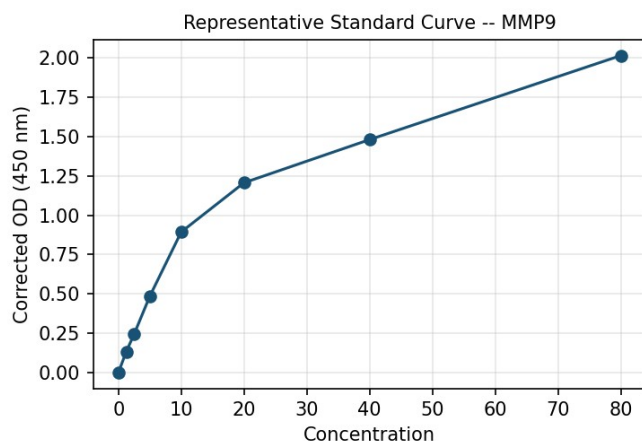
Specificity: This assay has high sensitivity and excellent specificity for detection of Human MMP9. No significant cross-reactivity or interference between Human MMP9 and analogues was observed.



This kit employs a sandwich enzyme immunoassay technique to quantify Human MMP9 in biological samples.

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SAMPLE STANDARD CURVE



Representative standard curve shown for illustration only. This curve should not be used to calculate unknown sample concentrations -- run a new standard curve with each assay.

ORDERING INFORMATION

Please visit www.affinityimmuno.com to order this product. Visa, Mastercard, AMEX and PayPal are accepted in our online store.

Your order will be processed immediately and you will be notified with a delivery timeframe.