



SensoLyte[®] MMP-13 ELISA Kit

Colorimetric

Revision Number: 1.2

Last updated: June 2012

Catalog #	72107
Kit Size	96 Assays in 96-well plate

- **Optimized Performance:** Optimal conditions for quantitative measurement of MMP-13.
- **Convenient Format:** All essential components to perform 96 assays in a 96-well plate format included.
- **Assured Reliability:** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	MMP-13 Microplate : pre-coated with anti-human MMP-13	1 96-well plate (8x12 strips)
Component B	20X Wash Buffer	25 mL
Component C	Recombinant Human MMP-13 Standards	2 vials
Component D	5X Assay Buffer	15 mL
Component E	Detection Antibody	2 vials
Component F	HRP-conjugated Streptavidin	200 µL
Component G	TMB Substrate Reagent	12 mL
Component H	Stop Solution	8 mL

Other Materials Required (but not provided)

- Colorimetric microplate reader: Capable of measuring absorbance at 450 nm.

Storage and Handling

- Store all kit components at –20°C.
- Component C should be stored at –80°C after reconstitution.
- Protect Component G from light.
- Component E can be stored at 4°C for 5 days after the addition of 1X assay buffer.

Introduction

The matrix metalloproteinases (MMPs) constitute a family of zinc-dependent endopeptidases that function within the extracellular matrix. These enzymes are responsible for the breakdown of connective tissues and are important in bone remodeling, menstrual cycle and the repair of tissue damage. MMP-13 (collagenase-3)¹ is a member of the MMP family of extracellular proteases. Targets of MMP-13 include collagen, gelatin, aggrecan, plasminogen and CXCL12. MMP-13 is secreted as a 60-kDa proenzyme (as measured by SDS-PAGE), and activated by cleavage to a mature 48-kDa MMP-13. MMP-13 is an important target for inhibitor screening due to its involvement in diseases such as cancer and arthritis.

The SensoLyte[®] MMP-13 ELISA Kit is a “sandwich” immunoassay for the quantitative determination of total human MMP-13 (pro and active forms) in serum, plasma, cell culture supernates and urine. This assay provides a 96-well plate (8x12 strips) coated with mouse anti-human monoclonal antibody, specific for capturing human MMP-13. After the addition of MMP-13 containing biological samples, biotinylated goat anti-human MMP-13 antibody is then added to the bound MMP-13. This is followed by sequential additions of enzyme-linked streptavidin and TMB substrate. A blue color develops in proportion to the amount of total MMP-13 bound. The reaction is stopped by the addition of sulfuric acid solution, changing the blue color to yellow. The intensity of signal is measured at 450 nm to determine the presence and quantity of MMP-13.

Protocol

Note: A standard curve must be run with each assay.

1. Prepare working solutions.

Note: Bring all reagents and samples to room temperature before starting the experiments. It is recommended to run samples at least in duplicate.

1.1 MMP-13 Microplate (Component A): Pre-coated with anti-human monoclonal antibody. Ready to use. Determine the number of wells needed and store the remainder at 4°C.

1.2 Wash Buffer: Dilute 20X Wash Buffer (Component B) to 1X with distilled water. (If wash buffer contains visible crystals, warm to room temperature and mix gently to dissolve).

1.3 Assay Buffer: Dilute 5X Assay Buffer (Component D) to 1X with distilled water. Use this 1X assay buffer to dilute or dissolve all standards, samples, and reagents.

Note: If precipitation occurs, resuspend before use.

1.4 Standard solution: Briefly spin down the vial of MMP-13 standard (Component C). Prepare 50 ng/mL of MMP-13 standard by adding 400 µL of 1X assay buffer to the vial. Dissolve the protein gently. Mix 80 µL of MMP-13 standard with 586.7 µL of 1X assay buffer to prepare a 6000 pg/mL stock solution. Pipette 400 µL of 1X assay buffer into each of six tubes. Perform six 3-fold serial dilutions by pipetting out 200 µL from the stock solution to get concentration of 2000, 666.7, 222.2, 74.07, 24.69, 8.23 pg/mL, and include a blank with 1X assay buffer only.

1.5 Detection Antibody: Briefly spin down the biotinylated goat anti-human MMP-13 (Component E). Dissolve the powder to prepare a concentrated solution by adding 100 µL of 1X assay buffer. Dilute the stock solution 80-fold with 1X assay buffer to obtain the working solution.

1.6 HRP-Streptavidin solution: Briefly spin down the HRP-Streptavidin (Component F), and gently pipette up and down to mix. Dilute HRP-Streptavidin (Component F) 700-fold with 1X assay buffer.

1.7 TMB Substrate Reagent (Component G): Chromogenic substrate, 3, 3', 5, 5'-tetramethylbenzidine (TMB) in buffered solution. Ready to use.

1.8 Stop solution (Component H): 2 M sulfuric acid. Ready to use.

2. Set up the reaction.

2.1 Add 100 µL of each standard and sample into the appropriate wells. Cover the wells and incubate on a shaker for 2.5 hours at room temperature or overnight at 4°C.

2.2 Discard the solution and wash 4 times with 200 µL of 1X wash buffer per well.

2.3 Add 100 µL of 1X prepared biotinylated antibody per well. Incubate for 1 hour on a shaker at room temperature.

2.4 Discard the solution and wash 4 times with 200 µL of 1X wash buffer per well.

2.5 Add 100 µL of prepared HRP-Streptavidin solution per well. Incubate for 45 minutes on a shaker at room temperature.

2.6 Discard the solution and wash 5 times with 200 µL of 1X wash buffer per well.

2.7 Add 100 µL of TMB Substrate Reagent (Component G) per well. Incubate for 30 minutes on a shaker at room temperature in the dark.

2.8 Add 50 µL of Stop Solution (Component H) per well. Read absorbance at 450 nm immediately.

Overview of Experimental Procedures:

- Prepare all reagents, samples, and standards according to the instructions



- Add 100 μ L standard or sample to each well
Incubate 2.5 hours at room temperature or overnight at 4°C
Discard solution and wash wells 4 times



- Add 100 μ L prepared biotin antibody to each well
Incubate 1 hour at room temperature
Discard solution and wash wells 4 times



- Add 100 μ L HRP-Streptavidin solution.
Incubate 45 minutes at room temperature
Discard solution and wash wells 5 times



- Add 100 μ L TMB Substrate reagent to each well
Incubate 30 minutes at room temperature



- Add 50 μ L Stop solution to each well
Read at 450 nm immediately

Appendix I. Data Analysis

1. Calculation of results:

- The absorbance reading from the blank control well is used as the background. This background reading should be subtracted from the readings of the other wells containing standards, controls and samples.
- Plot the standard curve with standard concentration on the x-axis and absorbance on the y-axis.

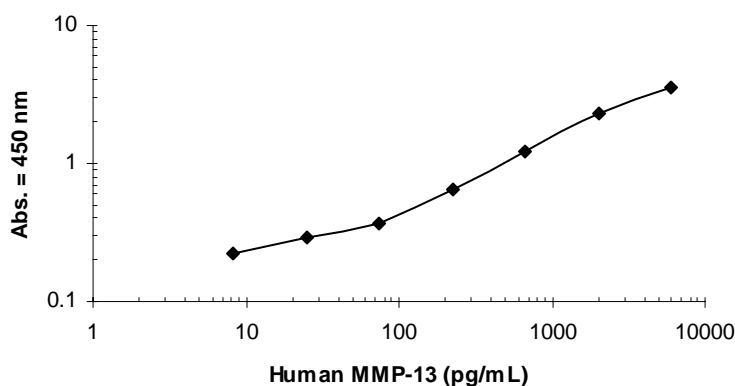


Figure 1. Human MMP-13 standard curve. Human MMP-13 was serially diluted in assay buffer, and absorbance recorded at 450 nm (EL-808, Bio-Tek Instruments).

2. Sensitivity:

The minimum detectable concentration of MMP-13 is typically less than 6 pg/mL.

3. Precision:

Intra-Assay: CV<10%

Inter-Assay: CV<12%

4. Recovery:

Recovery was determined by spiking various levels of human MMP-13 into human serum, plasma, and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	91.99	82-102
Plasma	93.13	83-103
Cell culture media	94.72	84-103

5. Linearity:

	Sample Type	Serum	Plasma	Cell culture media
1:2	Average % of Expected	92	93	94
	Range (%)	82-103	83-104	82-103
1:4	Average % of Expected	90	92	93
	Range (%)	83-102	84-103	83-102
1:8	Average % of Expected	94	91	92
	Range (%)	83-104	82-103	84-104

Appendix II. Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	1. Inaccurate pipetting 2. Improper standard dilution	1. Check accuracy of pipettes 2. Ensure a brief spin of Component C and dissolve the contents thoroughly by mixing gently.
Low signal	1. Too brief incubation 2. Inadequate reagent volumes or improper dilution	1. Ensure sufficient incubation time. Step 2.1 may be changed to overnight incubation. 2. Check pipettes and ensures correct preparations.
Large CV	1. Inaccurate pipetting	1. Check pipettes
High background	1. Plate is insufficiently washed 2. Contaminated wash buffer	1. Follow protocol for proper washing procedures. If using a plate washer, check that all ports are unobstructed. 2. Make fresh wash buffer
Low sensitivity	1. Improper storage of the ELISA kit 2. Stop solution	1. Store your standard at <-20°C after reconstitution, others at 4°C. Keep substrate solution protected from light 2. Stop solution should be added to each well before reading absorbance

Appendix III. Specificity

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested (eg., Angiopoietin-1, Angiostatin, B7-1, BMP-7, CD14, CD30, CD40, CD40 Ligand, CTLA-4, CXCL16, Dkk-4, DR6, Endostatin, E-Selectin, Follistatin, HB-EGF, HVEM, ICAM-2, IGF-IL, IL-10 Ra, IL-10 Rb, IL-18, IL-9, IL-2 Ra, IL-2 Rb, IL-5 Ra, LAP, LAP, L-Selectin, M-CSF R, MMP-1, 2, 3, 7, 8, 9, 10, and 12, PDGF-AB, SDF-1b, Tie-1, Tie-2, TIMP-3.)

Reference:

Freije, JM. et al. *J. Biol. Chem.* **269**, 16766 (1994).

Revised May 27, 2008