SensoLyte® Plus 520 MMP-13 Assay Kit

Fluorimetric and Enhanced Selectivity

Revision Number: 1.1	*Last updated: October 2014*
Catalog #	**AS-72019**
Kit Size	96 Assays in 96-well plate

- ***Optimized Performance:*** Optimal conditions for specifically detecting MMP-13 activity.
- ***Enhanced Value:*** Less expensive than the sum of individual components.
- ***Assured Reliability:*** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	Microplate coated with monoclonal anti human MMP-13 antibody	12 x 8 black strips
Component B	MMP-13 standard, recombinant human pro-MMP-13	10 µg/mL, 10µL
Component C	MMP dilution buffer	10 mL
Component D	10X Wash buffer	50 mL
Component E	APMA, 4-aminophenylmercuric acetate *Caution: Contain organic mercury. Dispose it according to your local regulations.*	100 mM, 150 µL
Component F	MMP-13 substrate 5-FAM/QXL™520 FRET peptide Ex/Em=490 nm/520 nm upon cleavage	50 µL
Component G	Assay buffer	50 mL
Component H	Stop Solution	10 mL
Component I	Adhesive cover strip	3 sheets

Other Materials Required (but not provided)

- Fluorescence microplate reader: Capable of detecting emission at 520 nm with excitation at 490 nm.

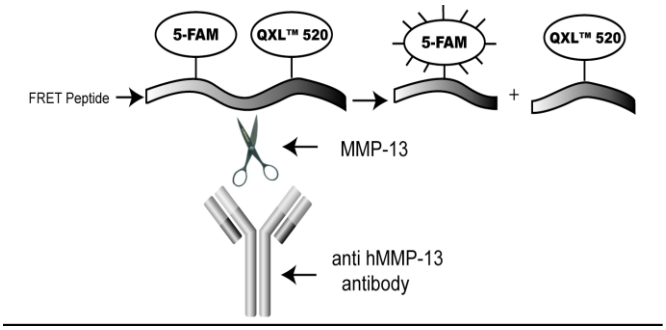
Storage and Handling

- Store all kit components, except Component B, at -20°C.
- Store Component B at -80°C.
- For convenience, Components D, G, H, I can be stored at RT.

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 pro-enzyme (as measured by SDS-PAGE), and activated by cleavage to a mature 48-kDa MMP-13. A 27 kDa C-terminal domain can be further removed by incubation with MMP-13 activators. MMP-13 is an important target for drug discovery due to its involvement in diseases such as cancer and arthritis.

The SensoLyte® Plus 520 MMP-13 Assay Kit is designed for specifically detecting MMP-13 activity in biological samples, such as culture medium, serum, plasma, synovial fluid, and tissue homogenate, which may contain multiple MMPs. Members of MMP family have poor substrate sequence specificity, making it difficult to use peptide substrate alone to differentiate the activity of a particular MMP from other MMPs. A monoclonal anti-human-MMP-13 antibody is therefore used in this kit to pull down both pro and active forms of MMP-13 from the mixture, and the activity of MMP-13 is quantified by a 5-FAM/QXL™520 fluorescence resonance energy transfer (FRET)[2] peptide (Scheme 1). Compared to a Mca/Dnp FRET substrate, this 5-FAM/QXL™520 substrate shows less interference from the autofluorescence of cellular components, and also provides better assay sensitivity. The assay can detect the activity of sub-nanogram of human MMP-13 without cross-reactions with human MMP-1, 2, 3, 7, 8, 9, 10, 12, and 14 (Figure 1, 2).



Scheme 1. The principle of SensoLyte® Plus 520 MMP-13 assay kit
MMP-13 in biological samples is captured by immobilized anti-MMP-13 antibody, and its proteolytic activity is measured by 5-FAM/QXL™520 FRET peptide. The fluorescence of 5-FAM (fluorophore) is quenched by QXL™520 (quencher) in the intact FRET peptide. Upon MMP-13 cleavage, the fluorescence of 5-FAM is recovered and can be monitored at Ex/Em=490±20 nm/520± 20 nm.

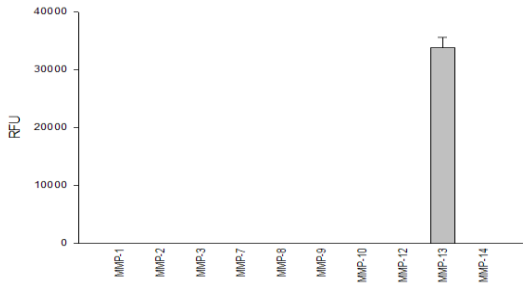


Figure 1. The specificity of SensoLyte® Plus 520 MMP-13 assay kit.
APMA-activated MMPs, 30 ng each, are added to the microplate pre-coated with anti-MMP-13 antibody. After incubation, the plate was washed and the activity of MMPs detected by 5-FAM/QXL™520 FRET peptide substrate. In 1h after adding the substrate, fluorescence signal was monitored at the excitation wavelength of 490 nm and emission wavelength of 520 nm, with cut off at 515 nm (FlexStation 384II). The reading from all wells was subtracted with the reading from blank control, which contains FRET substrate but no MMPs. (n=3, mean±S.D.)

Protocol

Note: Warm all the kit reagents to room temperature before use.

1. Prepare MMP-13 containing biological samples

1.1 Collect serum, plasma, synovial fluids or supernate of cell culture media (e.g. stimulated fibroblast) and centrifuge for 10-15 min at 1,000xg, 4°C. Collect the supernatant and store at -70°C until use.

1.2 Tissue samples should be homogenized in the assay buffer (Component G) containing 0.1% Triton-X 100, and then centrifuged for 15 min at 10,000xg at 4°C. Collect the supernatant and store at -70°C until use.

Note 1: Triton-X 100 is not provided.
Note 2: Biological samples can be further concentrated or diluted for the following experiment depending on the amount of MMPs in the sample. Please concentrate samples using a centrifugal filter (Millipore, Cat# 42407).
Note 3: During the collection of plasma, anticoagulants containing EDTA or citrate should be avoided. Heparin may be used as anticoagulant.

2. Pull down MMP-13 by antibody coated microplate

2.1 MMP-13 standard: Dilute MMP-13 standard (10 μg/mL, Component B) 50-fold in MMP dilution buffer (Component C) to get a concentration of 200 ng/mL. Then do six 2-fold serial dilutions in MMP dilution buffer. Prepare a blank control, which contains MMP dilution buffer only without MMP-13.

2.2 Add 100 μL/well samples, MMP-13 standards, and blank control to the microplate coated with monoclonal anti human MMP-13 antibody (Component A). Cover the plate with adhesive cover strip (Component I) to prevent evaporation. Incubate the plate on a plate shaker (40-100 rpm) at room temperature for 2 hrs.

2.3 Dilute 10X wash buffer (Component D) to 1X in deionized water. Wash the wells with 200 μL 1X wash buffer for four times.

3. Activate pro-MMP 13 by APMA

3.1 Dilute 100 mM APMA (Component E) in assay buffer (Component G) to 1 mM. Add 100 μL of 1 mM APMA per well. Cover the plate with adhesive cover strip (Component I) to prevent evaporation. Incubate the plate at 37°C for 40 min.

Note 1: Incubation must not exceed 40 min to prevent inactivation of MMP-13.
Note 2: APMA will activate all the pro-MMP-13 in your sample. If you want to measure endogenous active form of MMP-13 alone, this APMA activation step can be omitted.
Note 3: If 1 mM APMA looks cloudy, incubate it at 37°C water bath for 10-30 min.

3.2 Wash the wells with 200 μL 1X wash buffer for four times.

4. Measure MMP-13 activity by 5-FAM/QXL™ peptide 520 substrate

4.1 MMP-13 substrate solution: Dilute MMP-13 substrate (Component F) 200-fold in assay buffer (Component G).

4.2 Add 100 μL/well MMP-13 substrate solution to the plate.

4.3 Measure fluorescence signal:

For kinetic reading: Immediately start measuring fluorescence intensity at Ex/Em=490 nm/520 nm continuously and record data every 10 minutes for 60-120 minutes.

For end-point reading: Cover the plate with adhesive cover strip (Component I) to prevent evaporation. Incubate the reaction at room temperature in dark for 60 min to 16 h, then measure

fluorescence intensity at Ex/Em=490 nm/520 nm. Optional: 100 µL/well stop solution (Component H) can be added before taking the end-point reading.

5. Data analysis: Refer to appendix I.

Appendix I. Data Analysis

- The fluorescence reading from the blank control well represents the background fluorescence. Subtract this background reading from the readings of the other wells to get the relative fluorescence unit (RFU).
- For kinetics reading:
  - Plot data as RFU versus time for each sample.
  - Determine the slope of the linear portion of the data plot. Obtain the reaction velocity (V) in RFU/min or RFU/sec.
  - Plot V versus the concentration of MMP.
- For endpoint reading:
  - Plot data as RFU versus concentration of MMP (Figure 2).

  Note: TIMPs are able to bind and inactivate active form of MMP-13. The level of TIMPs in the biological samples may need to be determined in order to correctly interpret data.

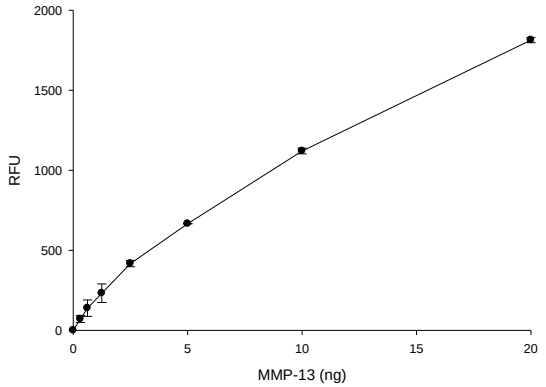


Figure 2. Sensitivity of the SensoLyte® Plus 520 MMP-13 assay.

Recombinant pro-MMP-13 was serially diluted and added to the plate coated with anti-MMP-13 antibody. Pro-MMP-13 was activated by APMA and its activity measured by cleavage of the 5-FAM/QXL™520 FRET peptide. Fluorescence signal was recorded with a filter set of Ex/Em =485±20 nm/ 528±20 nm (Bio-Tek FLx800). Endpoint reading (RFU) at 2h versus the amount of MMP-13 was plotted. The assay was able to detect as low as 0.3 ng enzyme

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Reference:

1. Freije, JM. et al. *J Biol Chem* **269**, 16766 (1994).
2. Stryer, L. *Annu Rev Biochem* **47**, 819 (1978).