SensoLyte® Plus 520 MMP-1 Assay Kit

Fluorimetric and Enhanced Selectivity

Revision number: 1.1	*Last updated: February 2015*
Catalog #	**AS-72012**
Kit Size	96 Assays in 96-well plate

- ***Optimized Performance:*** Optimal conditions for detecting MMP-1 activity in biological samples.
- ***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-1 antibody	12 X 8 black strips
Component B	MMP-1 standard, Proenzyme, Human Rheumatoid Synovial Fibroblast	10 µg/mL, 10 µL
Component C	MMP dilution buffer	10 mL
Component D	10 X Wash buffer	50 mL
Component E	APMA, 4-aminophenylmercuric acetate *Caution: Contains organic mercury. Dispose according to regulations.*	100 mM, 150 µL
Component F	MMP-1 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.

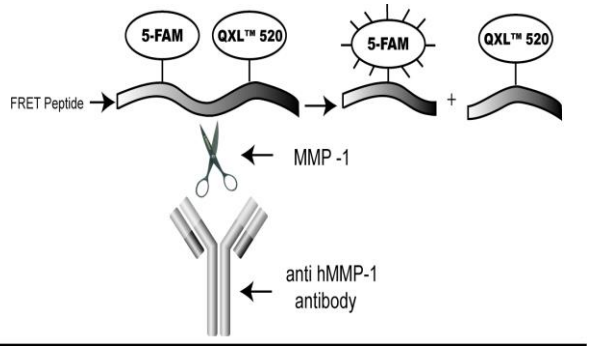
Storage and Handling

- 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

Matrix metalloproteinases (MMP's) belong to a family of secreted or membrane-associated zinc endopeptidases capable of digesting extracellular matrix components[1,2]. MMP-1 (collagenase) is involved in tumor development and metastasis [3,4] and rheumatoid arthritis[5]. It is proposed as a therapeutic target for these diseases. MMP-1 is secreted as pro-enzyme, which consists of a propeptide of 80 amino acids, a catalytic domain of 162 amino acids, a 16-residue linker region, and a hemopexin domain of 189 amino acids[6]. The pro-MMP-1 consists of a major Mr of 52-kDa unglycosylated and a minor Mr of 57-kDa glycosylated form. Proteolytic activation of the 57- and 52-kDa species forms 47- and 42-kDa active collagenase[6].

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



Scheme 1. The principle of SensoLyte® Plus 520 MMP-1 assay kit.
MMP-1 in biological samples is captured by immobilized anti-MMP-1 antibodies, 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-1 cleavage, the fluorescence of 5-FAM is recovered and can be monitored at Ex/Em=490±20 nm/520± 20 nm.

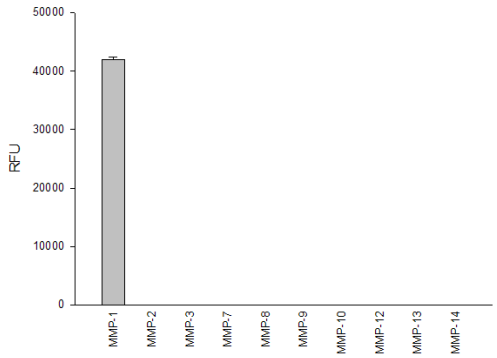


Figure 1. The specificity of SensoLyte® Plus 520 MMP-1 assay kit.
APMA-activated MMPs, 30 ng each, are added to the microplate pre-coated with anti-MMP-1 antibody. After incubation, the plate was washed and the activity of MMPs detected by 5-FAM/QXL™520 FRET peptide substrate. In 1h after adding the substrate, the fluorescence signal was read (FlexStation 384II), at the excitation wavelength of 490 nm and emission wavelength of 520 nm, with cut off at 515 nm. 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 of the kit reagents to room temperature before use.

1. Prepare MMP-1 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 1000 X g, 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% (v/v) Triton-X 100, and then centrifuged for 15 min at 10,000x g 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 step depending on the amount of MMPs present in the sample. 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-1 by antibody coated microplate

2.1 MMP-1 standard: Dilute MMP-1 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-1.

2.2 Add 100 µL/well samples, MMP-1 standards, and blank control to the microplate coated with monoclonal anti human MMP-1 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 four times.

3. Activate pro-MMP-1 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 3 hrs.

Note 1: APMA will activate all the pro-MMP-1 in your sample. If you want to measure endogenous active form of MMP-1 alone, this APMA activation step can be omitted.
Note 2: If 1 mM APMA looks cloudy, incubate in a 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-1 activity by 5-FAM/QXL™ peptide 520 substrate

4.1 MMP-1 substrate solution: Dilute MMP-1 substrate (Component F) 200-fold in assay buffer (Component G).

4.2 Add 100 µL/well MMP-1 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 the dark for 60 min to 16h, 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 the active form of MMP-1. The level of TIMPs in the biological samples may have to be determined in order to correctly interpret the data.

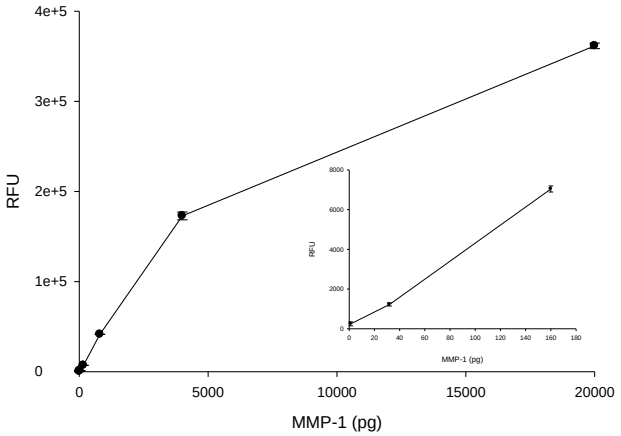


Figure 2. Sensitivity of the SensoLyte Plus® 520 MMP-1 assay.

Recombinant pro-MMP-1 was serially diluted and added to the plate coated with anti-MMP-1 antibody. Pro-MMP-1 was activated by APMA and its activity was measured by the cleavage of 5-FAM/QXL™520 FRET peptide. Fluorescence signal was with the excitation at 490 nm and emission at 520 nm, cut off at 515 nm (FlexStation384II). Endpoint reading (RFU) at 2h versus the amount of MMP-1 was plotted. The reading at the low end of enzyme amount is shown in the insert. The assay was able to detect as low as 30 pg enzyme (n=3, mean±S.D.).

References

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7. Stryer, L. *Annu Rev Biochem* **47**, 819 (1978).