SensoLyte® Plus 520 MMP-9 Assay Kit

Fluorimetric and Enhanced Selectivity

Revision Number: 1.1	*Last updated: October 2014*

Catalog #	**AS-72017**
Kit Size	96 Assays in 96-well plate

- ***Optimized Performance:*** Optimal conditions for detecting MMP-9 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-9 antibody	12 X 8 black strips
Component B	MMP-9 standard, recombinant human proMMP-9	10 µg/mL, 10 µL
Component C	MMP dilution buffer	5 mL
Component D	10 X 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-9 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.

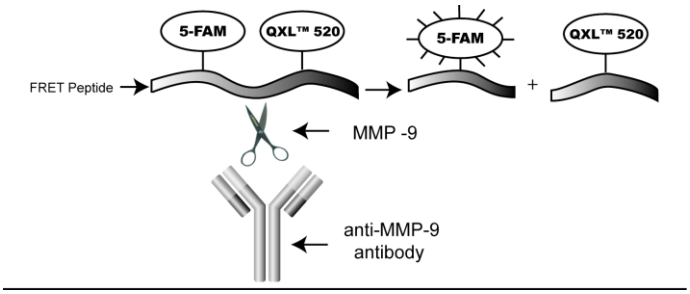
Storage and Handling

- 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 (MMPs) belong to a family of secreted or membrane-associated zinc endopeptidases capable of digesting extracellular matrix components[1,2]. The importance of MMPs in tumor development and invasion as well as other diseases is well known. MMP-9 (92-kDa gelatinase, collagenase-IV)[3,4] is involved in a number of diseases such as cancer, angiogenesis, alopecia, and metastasis. It is proposed as a therapeutic target for these diseases. Latent 92 kDa MMP-9 can be activated to the 82 and 65 kDa active forms.

The SensoLyte® Plus 520 MMP-9 Assay Kit is designed for specifically detecting MMP-9 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-9 is therefore used to pull down both pro and active forms of MMP-9 from the mixture first, and the activity of MMP-9 is then quantified using a 5-FAM/QXL™520 fluorescence resonance energy transfer (FRET)[5] 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 sub-nanogram level of active human MMP-9 without cross-reactions with human MMP-1, 2, 3, 7, 8, 10, 12, 13, and 14 (Figure 1).



Scheme 1. The principle of SensoLyte® Plus 520 MMP-9 assay kit.
MMP-9 in biological samples is captured by immobilized MMP-9 antibodies, and its proteolytic activity is measured by the 5-FAM/QXL™520 FRET peptide. Fluorescence of 5-FAM (fluorophore) is quenched by QXL™520 (quencher) in the intact FRET peptide. Upon MMP-9 cleavage, the fluorescence of 5-FAM is recovered and can be monitored at Ex/Em=490±20 nm/520± 20 nm.

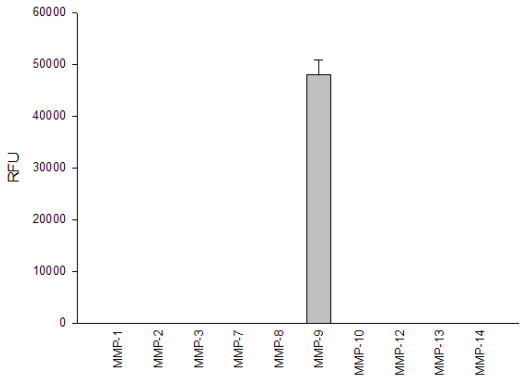


Figure 1. The specificity of SensoLyte® Plus 520 MMP-9 assay kit.
APMA-activated MMPs, 30 ng each, are added to the microplate pre-coated with anti-MMP-9. After incubation, the plate was washed and the activity of MMPs detected by adding 5-FAM/QXL™520 FRET peptide substrate. The fluorescence signal was monitored in 1 hr after adding the substrate at the excitation wavelength of 490 nm and emission 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-9 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 1000X 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% 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 provided by the customer.
Note 2: Biological samples can be further concentrated or diluted for the next step depending on the amount of MMPs in the sample. Samples can be concentrated by 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-9 by antibody coated microplate

2.1 MMP-9 standard: Add 10 µL MMP-9 standard (10 µg/mL, Component B) to 490 µL 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-9.

Note: Do not vortex the enzyme solution! Keep enzyme on ice before use.

2.2 Add 100 µL/well samples, MMP-9 standards, and blank control to the microplate coated with monoclonal anti-human MMP-9 (Component A). Cover the plate with adhesive cover strip (Component I) to prevent evaporation. Incubate the plate on a plate shaker (100-200 rpm) at room temperature for 1 hr.

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 9 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 2 hrs.

Note 1: APMA will activate all the pro-MMP-9 in your sample. If you want to measure endogenous active form of MMP-9 alone, this APMA activation step can be omitted.
Note 2: The MMP-9 standard contains mainly zymogen. APMA activation must be performed.
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-9 activity by 5-FAM/QXL™ peptide 520 substrate

4.1 MMP-9 substrate solution: Dilute MMP-9 substrate (Component F) 200-fold in assay buffer (Component G).

Table 1. MMP-9 substrate solution for one 96-well plate (100 assays)

Components	Volume
MMP-9 substrate (Component F)	50 µL
Assay buffer (Component G)	10 mL
Total volume	10 mL

4.2 Add 100 µL/well MMP-9 substrate solution to all wells, including MMP-9 standard, samples, and blank control.

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 min.

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 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-9. The level of TIMPs in the biological samples may need to be determined in order to correctly interpret the data.

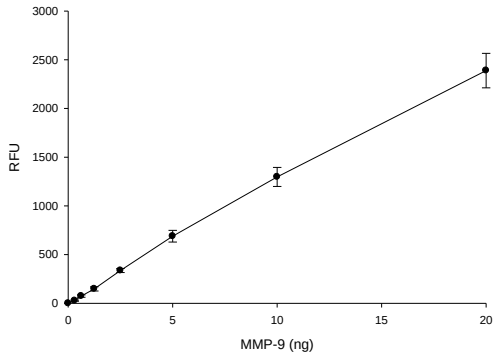


Figure 2. Sensitivity of the SensoLyte® Plus 520 MMP-9 assay.

Recombinant pro-MMP-9 was serially diluted and added to plate coated with anti-MMP-9 antibody. Pro-MMP-9 was activated by APMA and its activity measured by the 5-FAM/QXL™520 FRET peptide. Fluorescence signal was monitored with a filter set of excitation/emission=485±20 nm/528±20 nm (Bio-Tek FLx800). Endpoint reading (RFU) at 2h versus the amount of MMP-9 was plotted. The assay was able to detect as low as 0.3 ng of enzyme. (n=3, mean±S.D.)

References

1. Woessner, JF. Jr. et al. *J Biol Chem* **263**, 16918 (1988).
2. Woessner, JF. Jr. *FASEB J* **5**, 2145 (1991).
3. Wilhelm, SM. et al., *J Biol Chem* **264**, 17213 (1989).
4. Fosang, AJ. et al. *Biochem J* **295**, 273 (1993).
5. Stryer, L. *Annu Rev Biochem* **47**, 819 (1978).