SensoLyte® Plus 520 MMP-2 Assay Kit
Fluorimetric and Enhanced Selectivity

Revision Number: 1.1	*Last updated: October 2014*

Catalog #	**AS-72224**
Kit Size	96 Assays in 96-well plate

- ***Optimized Performance:*** Optimal conditions for specifically detecting MMP-2 activity.
- ***Enhanced Value:*** It provides enough reagents to perform 100 assays in a 96-well format.
- ***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-2 antibody	12 x 8 black strips
Component B	MMP-2 standard, recombinant human pro-MMP-2	10 µg/mL, 15µL
Component C	Assay buffer	50 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-2 substrate 5-FAM/QXL™520 FRET peptide Ex/Em=490 nm/520 nm upon cleavage	50 µL
Component G	Stop Solution	10 mL
Component H	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, and H can be stored at room temperature (RT).

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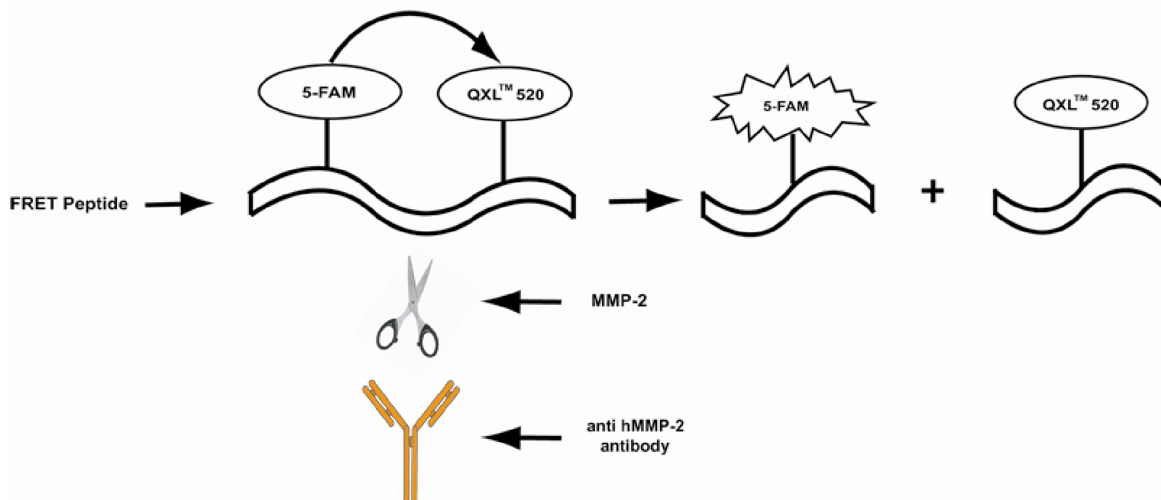
Introduction

Matrix metalloproteinases (MMPs) belong to a family of secreted or membrane-associated zinc endopeptidases capable of digesting extracellular matrix components.[1, 2] MMP-2 (72-kDa gelatinase-A/type IV collagenase) is responsible for degradation of collagen, fibronectin, laminin, and elastin.[3, 4] It has been shown that MMP-2 plays a key role in angiogenesis, tumor cell invasion, and metastasis.[5-7] MMP-2 is also considered a therapeutic target for cancer.[8, 9]

The SensoLyte® Plus 520 MMP-2 Assay Kit is designed specifically for detecting MMP-2 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, thus using peptide substrate alone to differentiate the activity of a particular MMP from other MMPs is inadequate. A monoclonal anti-human-MMP-2 antibody is therefore employed to pull down MMP-2 from the mixture. MMP-2 activity is then quantified by a 5-FAM/QXL™ 520 fluorescence resonance energy transfer (FRET) peptide (Scheme 1). The long wavelength fluorescence of 5-FAM is less interfered by the autofluorescence of components in biological samples and test compounds. The assay can detect as low as 3 ng/mL active MMP-2 enzyme. The assay has no cross-reactivity with human MMP-1, 3, 7, 8, 9, 10, 12, 13, 15, 16 and has minimal cross-reactivity with MMP-14 (Fig.1).



Scheme 1. The principle of SensoLyte® Plus 520 MMP-2 assay kit
MMP-2 in biological samples is captured by pre-coated anti-MMP-2 antibody, and its proteolytic activity 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-2 cleavage, the fluorescence of 5-FAM is recovered and can be monitored at Ex/Em=490±20 nm/520± 20 nm.

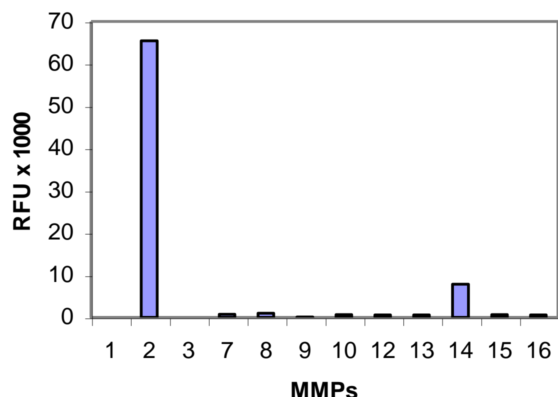


Figure 1. Specificity of SensoLyte® Plus 520 MMP-2 assay kit.
APMA-activated MMPs, 30 ng each, are added to the microplate pre-coated with anti-MMP-2 antibody. After incubation, the plate was washed and the activity of MMPs detected by 5-FAM/QXL™520 FRET peptide substrate. 18 hrs after adding the substrate, fluorescence signal was monitored at Ex/Em=490/520 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: Bring all the kit reagents to room temperature before use.

1. Prepare MMP-2 containing biological samples

1.1 Collect serum, plasma, synovial fluids or supernate of cell culture media and centrifuge for 10-15 min at 1,000x 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 C) containing 0.1% Triton-X 100, and then centrifuged for 15 min at 10,000x g, 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 experiment depending on the amount of MMPs in the sample. Concentrate samples using a centrifugal filter (Millipore, Cat# UFC905096).
Note 3: During the collection of plasma, anticoagulants containing EDTA or citrate should be avoided. Heparin can be used as anticoagulant.

2. Activate pro-MMP-2 by APMA:

2.1 MMP-2 standard: Dilute MMP-2 standard (10 µg/mL, Component B) 50-fold in assay buffer (Component C) to get a concentration of 200 ng/mL. Incubate pro-MMP-2 with 1 mM APMA for 1 h at 37°C. Activate pro-MMP-2 immediately before the experiment.

2.2 Incubate biological samples with 1 mM APMA for 1 h at 37°C to activate pro-MMP-2. APMA will activate all the pro-MMP-2 in your samples. If measuring endogenous active form of MMP-2 alone, APMA activation step can be omitted.

Note 1: Keep activated enzyme on ice. Avoid vigorously vortexing the enzyme. Prolonged storage of activated enzyme will further de-activate the enzyme.
Note 2: APMA belongs to the organic mercury class of compounds and must be handled with care! Dispose according to appropriate regulations.
Note 3: Activation of zymogen by APMA at higher protein concentration is preferred. After activation, the enzyme can be further diluted.

3. Prepare MMP-2 standard and samples

3.1 Dilute activated MMP-2 standard from step 2.1: use six 2-fold serial dilutions in assay buffer. Prepare a blank control, which contains assay buffer only without MMP-2.

3.2 MMP-2 biological samples: Dilute APMA activated samples as needed.

4. Pull down MMP-2 by antibody coated microplate

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

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

5. Measure MMP-2 activity by 5-FAM/QXL™ 520 peptide substrate

5.1 MMP-2 substrate solution: Dilute MMP-2 substrate (Component F) 200-fold in assay buffer (Component C). Refer to Table 1. If not using the entire plate, dilute only the amount needed for the experiment.

Table 1. Substrate solution for one 96-well plate (100 assays)

Components	Volume
MMP-2 substrate (Component F)	50 µL
Assay buffer (Component C)	9950 µL
Total volume	10 mL

5.2 Add 100 µL/well diluted MMP-2 substrate solution to the plate.

5.3 Measure fluorescence signal:

Cover the plate with adhesive cover strip (Component H) to prevent evaporation. Incubate the reaction at RT in dark for 1 h to 24 hrs, then measure fluorescence intensity at Ex/Em=490 nm/520 nm. Optional: 100 µL/well stop solution (Component G) can be added before taking the end-point reading.

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

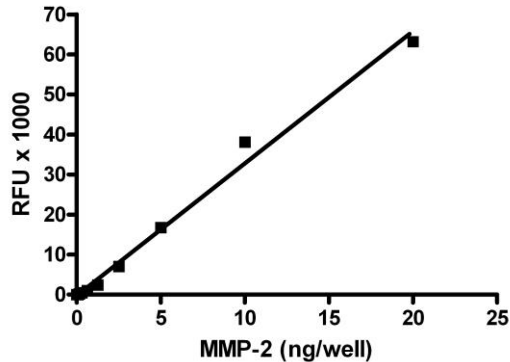


Figure 2. Sensitivity of the SensoLyte® Plus 520 MMP-2 assay.

Recombinant pro-MMP-2 was activated by APMA, and then serially diluted and added to the plate pre-coated with anti-MMP-2 antibody. Its activity was measured by cleavage of the 5-FAM/QXL™ 520 FRET peptide. Fluorescence signal was monitored at Ex/Em =490/520 nm (FlexStation 384II). Endpoint reading (RFU) at 18 hrs versus the amount of MMP-2 was plotted.

Reference:

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