SensoLyte® 520 MMP Profiling Kit
Fluorimetric

Revision# 1.1	*Last Updated: May 2019*
Catalog #	**AS-71136**
Kit Size	Two 96-well plates

- ***Convenient Format:*** All essential assay components are included.
- ***Optimized Performance:*** Optimal conditions for the screening of the best peptide substrate for MMPs.
- ***Enhanced Value:*** Less expensive than the sum of individual components.
- ***High Speed:*** Minimal hands-on time.
- ***Assured Reliability:*** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	Microplate pre-coated with 16 different MMP substrates. Peptide sequence: Refer to **Appendix I** Table 2. Ex/Em=490 nm/520 nm upon cleavage	2 identical 96-well plates
Component B	5-FAM-Pro-Leu-OH, fluorescence reference standard Ex/Em=490 nm/520 nm	1 mM, 10 µL
Component C	APMA, 4-aminophenylmercuric acetate *Caution: Toxic! Handle with care.*	1 M, 100 µL
Component D	Assay buffer	40 mL
Component E	Stop solution	20 mL

Other Materials Required (but not provided)

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

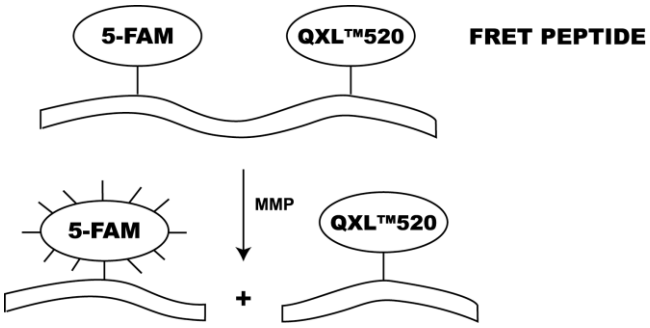
Storage and Handling

- Store kit components at -20°C
- Protect Components A and B from light and moisture
- Components D and E can be stored at room temperature for convenience.

Introduction

Matrix metalloproteinases (MMPs) belong to a family of secreted or membrane-associated zinc endopeptidases capable of digesting extracellular matrix components[1;2]. These enzymes are responsible for connective tissues breakdown, bone remodeling, and damaged tissue repair. They are also involved in a number of diseases such as tumor development and metastasis[3-6] as well as in rheumatoid arthritis[7;8]. They are proposed as the therapeutic target for these diseases.

The SensoLyte® MMP Profiling Kit contains two 96-well plates pre-coated with sixteen different FRET peptide substrates (**Appendix I**, Table 2).[9] It provides a convenient platform for profiling substrate specificity for MMPs. 5-FAM and QXL™520, a pair of optimal fluorophore and quencher, are used in these FRET peptides. In an intact FRET peptide, the fluorescence of 5-FAM is quenched by QXL™520. Upon MMP cleavage of the FRET peptides into two separate fragments, the fluorescence of 5-FAM is recovered, and can be monitored at excitation/emission = 490 nm/520 nm (**Scheme 1**). With excellent fluorescence quantum yield and longer excitation and emission wavelength, the fluorescence signal of 5-FAM is less interfered by the autofluorescence of test compounds and cellular components, thus providing better assay sensitivity.



Scheme 1. Proteolytic cleavage of the 5-FAM/QXL™520 paired FRET peptide.

Protocol

Note 1: Activate MMP (zymogen) by treating with 1 mM APMA (diluted Component C) in assay buffer (Component D). For detailed procedures, please refer to **Appendix II, Table 3**. Prepare freshly activated MMPs for each experiment.
Note 2: Warm all the kit components until thawed to room temperature before starting the experiments. Store activated MMPs at 4°C or on ice before the experiment.

1. Prepare working solutions.

1.1 Prepare MMP substrate plate: Add 50 µL assay buffer (Component D) to wells in Columns 3 to 12 on the pre-coated plate (Component A, refer to **Table 1**). Leave Columns 1 and 2 empty for setting up the fluorescence reference standard later. Completely dissolve the substrate by shaking the plate on a plate shaker at 100-200 rpm for 5 minutes.

Table 1. The layout of FRET peptide substrates on the 96-well microplate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	RS1	RS1	SB 1	SB 1	SB 1	SB 1	SB 1	SB 9	SB 9	SB 9	SB 9	SB 9
B	RS2	RS2	SB 2	SB 2	SB 2	SB 2	SB 2	SB 10	SB 10	SB 10	SB 10	SB 10
C	RS3	RS3	SB 3	SB 3	SB 3	SB 3	SB 3	SB 11	SB 11	SB 11	SB 11	SB 11
D	RS4	RS4	SB 4	SB 4	SB 4	SB 4	SB 4	SB 12	SB 12	SB 12	SB 12	SB 12
E	RS5	RS5	SB 5	SB 5	SB 5	SB 5	SB 5	SB 13	SB 13	SB 13	SB 13	SB 13
F	RS6	RS6	SB 6	SB 6	SB 6	SB 6	SB 6	SB 14	SB 14	SB 14	SB 14	SB 14
G	RS7	RS7	SB 7	SB 7	SB 7	SB 7	SB 7	SB 15	SB 15	SB 15	SB 15	SB 15
H	RS8	RS8	SB 8	SB 8	SB 8	SB 8	SB 8	SB 16	SB 16	SB 16	SB 16	SB 16

Note: RS=Reference standard, SB=Substrates 1 to 16. Please refer to Appendix I Table 2 for substrate sequences.

1.2 5-FAM-Pro-Leu-OH fluorescence reference standard: Dilute 1 mM 5-FAM-Pro-Leu-OH (Component B) to 5 μM in assay buffer (Component D). Do 2-fold serial dilutions to get concentrations of 2.5, 1.25, 0.63, 0.31, 0.16, 0.08, 0.04 μM. Add 50 μL of 5-FAM-Pro-Leu-OH solution from 5 μM to 0.04 μM into the reference standard wells, RS1 through to RS8.

1.3 MMP diluent: Dilute activated MMP to an appropriate concentration in assay buffer (Component D). Each well in a 96-well microplate will require 50 μL of diluted enzyme.

Note 1*:* Pro-MMP needs to be activated by APMA before use. Avoid vigorous vortexing of enzyme.
Note 2: APMA belongs to organic mercury. Handle with care! Do not dump into sink!

2. Calibrate the fluorescence microplate reader.

2.1 Add 50 μL of assay buffer to the reference standard wells. Mix the reagents by shaking the plate gently for 30 seconds.

2.2 Measure the fluorescence intensity of the reference standard at Ex/Em=490 nm/520 nm. Adjust the sensitivity of the microplate reader until satisfactory signals can be read. Use the same setting of sensitivity in subsequent enzymatic reactions.

3. Initiate the enzymatic reaction.

3.1 Add 50 μL assay buffer into the selected substrate-containing wells to serve as substrate controls. The fluorescence reading from the substrate control well is the background fluorescence.

3.2 Add 50 μL MMP diluent into the rest of the substrate-containing wells. Mix the reagents by shaking the plate gently for 30 seconds.

3.3 Measure fluorescence signal:

- For kinetic reading: Immediately start measuring fluorescence intensity at Ex/Em=490 nm/520 nm continuously and record data every 5 minutes for 30 to 60 minutes.
- For end-point reading: Incubate the reaction at room temperature for 30 to 60 minutes. Keep the plate from direct light. Optional: Add 50 μL/well stop solution (Component E). Mix the reagents, then measure fluorescence intensity at Ex/Em=490 nm/520 nm.

Perform Data Analysis.

- The fluorescence reading from the substrate control wells is the background fluorescence. This background reading should be subtracted from the readings of the other wells.
- Plot the 5-FAM-Pro-Leu-OH fluorescent reference standard curve as RFU (relative fluorescent units) versus concentration as shown in Figure 1.

  Note: The final concentration of 5-FAM-Pro-Leu-OH reference standard solutions are 2.5, 1.25, 0.63, 0.31, 0.16, 0.08, 0.04, and 0.02 μM. This reference standard is used to calibrate for the variation of different instruments and for the different batches of experiments. It is also an indicator of the amount of MMP enzymatic reaction final product.
- Optional: If the stop solution (Component E) was added into the enzymatic reaction before taking the end-point reading, the same volume of stop solution should be added to reference standard wells for better comparison.
- Plot data as RFU versus time for each sample.
- Determine the range of initial time points during which the reaction is linear. 10-15% conversion appears to be the optimal range.
- Obtain the initial reaction velocity (V_0) in RFU/min or μM/min. Determine the slope of the linear portion of the data plot.
- A variety of data analyses can be done, e.g., determining inhibition %, IC_{50}, K_m, K_i, etc.

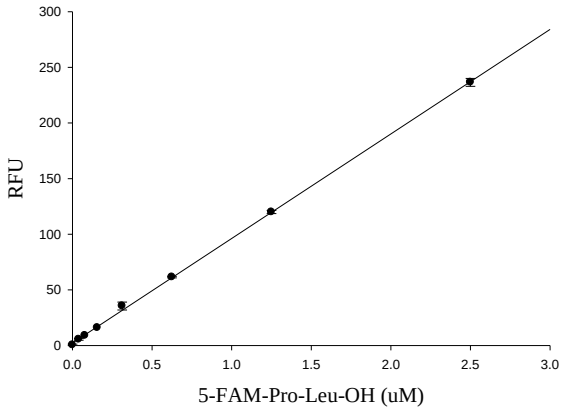


Figure 1. 5-FAM-Pro-Leu-OH reference standard. 5-FAM-Pro-Leu-OH was serially diluted in assay buffer according to the protocol. The fluorescence was monitored by a microplate reader (Bio-Tek FLx800) with a filter set of Ex/Em=485±20 nm/528±20 nm. (mean±S.D., n=2).

Appendix I Table 2: MMP substrate sequences in the 96-well plate.

Catalog Number	Cleavage Enzyme	FRET Peptide Sequence
SB1	MMP-2/13/14/16	QXL520™-Pro-Leu-Gly-Leu-Trp-Ala-D-Arg-Lys(5-FAM)-NH_2[1-3],
SB2	MMP-1/7/8/12/13	QXL520™ -Pro-Leu-Ala-Leu-Trp-Ala-Arg-Lys(5-FAM)-NH_2[2;4;5],
SB3	MMP-2/8/10//13/14/16/24	QXL520 ™ -Pro-Leu-Gly-Cys(Me)-His-Ala-D-Arg-Lys(5-FAM)-NH_2[6]
SB4	MMP-1/2/8/10/13/14/16	5-FAM-Pro-Leu-Ala-Nva-Dap(QXL520 ™)-Ala-Arg-NH_2[7-9]
SB5	MMP-1/2/7/8/12/13	5-FAM-Pro-Leu-Gly-Leu-Dap(QXL520 ™)-Ala-Arg-NH_2[1;10]
SB6	MMP-2/13	QXL520 ™ -Pro-Leu-Gly-Met-Trp-Ser-Arg-Lys(5-FAM)-NH_2[1;11]
SB7	MMP-7/12/13	QXL520 ™ -Pro-Tyr-Ala-Tyr-Trp-Met-Arg-Lys(5-FAM)-NH_2[12;13]
SB8	MMP-2/3/7/10/12/13/14	QXL520 ™ -Arg-Pro-Lys-Pro-Leu-Ala-Nva-Trp-Lys(5-FAM)-NH_2[12;14]
SB9	MMP-1/2/7/8/12/13	QXL520 ™ -Arg-Pro-Leu-Ala-Leu-Trp-Arg-Lys(5-FAM)-NH_2[15-17]
SB10	MMP-13	QXL520 ™ -Pro-Leu-Ala-Tyr-Trp-Ala-Arg-Lys(5-FAM)-NH_2[1;18]
SB11	MMP-2/7/8/13/14/16/24	5-FAM-Pro-Cha-Gly-Nva-His-Ala-Dap(QXL520 ™)-NH_2[19;20]
SB12	MMP-1/2/3/12/13	5-FAM-Arg-Pro-Lys-Pro-Tyr-Ala-Nva-Trp-Met-Lys(QXL520 ™)-NH_2[5;21]
SB13	MMP-3/12	5-FAM-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Lys(QXL520 ™)-NH_2[5;21]
SB14	MMP-1/2/3/7/8/9/10/12/13/14/15/16/24	QXL520 ™ -γ-Abu-Pro-Cha-Abu-Smc-His-Ala-Dab(5-FAM)-Ala-Lys-NH_2[22] (Smc=S-Methyl-L-cysteine)
SB15	MMP-2/7/12/13/14/16	QXL52 ™ -γ-Abu-Pro-Gln-Gly-Leu-Dab(5-FAM)-Ala-Lys-NH_2[23]
SB16	MMP-12/13	QXL520 ™ -Arg-Pro-Lys-Pro-Gln-Gln-Phe-Trp-Lys((5-FAM)-NH_2[14]

Appendix II

Table 1: Protocols for pro-MMP activation.

MMPs	Activated by treating with
MMP-1 (collagenase)	1 mM APMA (diluted Component C) at 37°C for 3 h.
MMP-2 (gelatinase)	1 mM APMA (diluted Component C) at 37°C for 0-20 min.
MMP-3 (stromelysin)	1 mM APMA (diluted Component C) at 37°C for 24 h.
MMP-7 (matrilysin, PUMP-1)	1 mM APMA (diluted Component C) at 37°C for 20 min-1 h.
MMP-8 (neutrophil collagenase)	1 mM APMA (diluted Component C) at 37°C for 1 h.
MMP-9 (92 kDa gelatinase)	1 mM APMA (diluted Component C) at 37°C for 2 h.
MMP-10 (stromelysin 2)	1 mM APMA (diluted Component C) at 37°C for 24 h.
MMP-11 (stromelysin-3)	Already in active form. No APMA treatment is necessary.
MMP-12 (macrophage elastase)	1 mM APMA (diluted Component C) at 37°C for 2 h.
MMP-13 (collagenase-3)	1 mM APMA (diluted Component C) at 37°C for 40 min.
MMP-14	1 mM APMA (diluted Component C) at 37°C for 2-3 h.

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