



## EnzoLyte™ 520 MMP-12 Assay Kit \*Fluorimetric\*

<b>Catalog #</b>	<b>71157</b>
<b>Unit Size</b>	1 Kit
<b>Kit Size</b>	100 Assays (96-well) or 250 Assays (384-well)

This kit is optimized to detect MMP-12 activity using a 5-FAM/QXL™520 FRET peptide as a substrate with its fluorescence monitored at Ex/Em=490 nm/520 nm upon proteolytic cleavage. This substrate shows excellent specificity to MMP-12 and minimal cross-reaction with MMP-1, 2, 7, 8, 9, 13 and 14. Ample materials are provided to perform 100 assays in a 96-well format or 250 assays in a 384-well format. The kit has the following features:

- **Convenient Format:** All essential assay components are included.
- **Optimized Performance:** Optimal conditions for the detection of MMP-12 activity.
- **Enhanced Value:** Less expensive than the sum of individual components.
- **High Speed:** Minimal hands-on time.
- **Assured Reliability:** Detailed protocol and references are provided.

### USA and Canada Ordering Information

#### **AnaSpec Corporate Headquarter**

2149 O'Toole Ave.  
 San Jose, CA 95131  
 Toll-Free: 800-452-5530  
 Tel: 408-452-5055  
 Fax: 408-452-5059  
 E-mail: [service@anaspec.com](mailto:service@anaspec.com)  
 Internet: [www.anaspec.com](http://www.anaspec.com)

### International Ordering Information

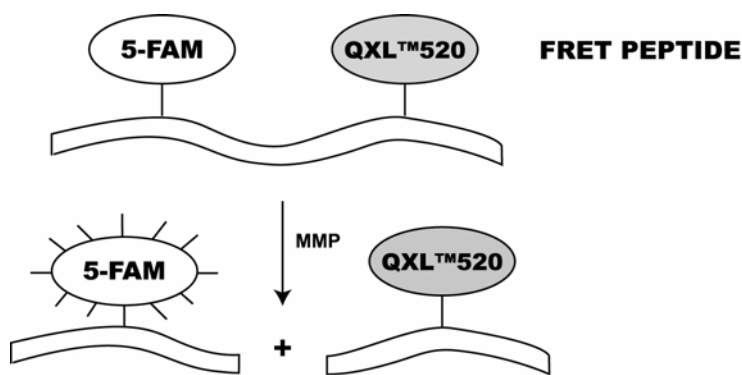
A list of international distributors is available at [www.anaspec.com](http://www.anaspec.com).

## **INTRODUCTION**

Matrix metalloproteinases (MMPs) belong to a family of secreted or membrane-associated zinc endopeptidases capable of digesting extracellular matrix components<sup>1,2</sup>. MMP-12 (macrophage elastase) is involved in smoke-induced emphysema<sup>3</sup>, tumor<sup>4</sup> and other diseases. MMP-12 is secreted as a 54 kDa zymogen and becomes the mature 45 kDa active form after proteolytic cleavage.

The EnzoLyte™ 520 MMP-12 Assay Kit provides a convenient assay for high throughput screening of MMP-12 inducers and inhibitors. It detects MMP-12 activity in a variety of biological samples using a 5-FAM/QXL™520 fluorescence resonance energy transfer (FRET)<sup>5</sup> peptide. In the intact FRET peptide, the fluorescence of 5-FAM is quenched by QXL™520. Upon cleavage into two separate fragments by MMP-12 (**Scheme 1**), the fluorescence of 5-FAM is recovered, and can be monitored at excitation/emission wavelengths = 490 nm/520 nm. With excellent fluorescence quantum yield and longer wavelength, 5-FAM shows less interference from autofluorescence of test compounds and cellular components. Compared to an EDANS/DABCYL FRET substrate, this 5-FAM/QXL™520 substrate provides better assay sensitivity.

The assays are performed in a convenient 96-well or 384-well microplate format.



**Scheme 1.** Proteolytic cleavage of 5-FAM/QXL™520 FRET peptide by MMPs.

## **KIT COMPONENTS, STORAGE AND HANDLING**

*Note: Store all kit components at -20 °C, and keep components A and B away from light. If used frequently, components D and E can be stored at 4 °C for convenience.*

- Component A:** MMP-12 substrate (60 µL)  
5-FAM/QXL™520 FRET peptide  
Ex/Em=490 nm/520 nm upon cleavage
- Component B:** 5-FAM-Pro-Leu-OH, fluorescence reference standard (1 mM, 10 µL)  
Ex/Em=490 nm/520 nm
- Component C:** APMA, 4-aminophenylmercuric acetate (1 M, 20 µL)  
Caution: Organic mercury. Handle with care! Do not dump into sink!
- Component D:** Assay buffer (20 mL)
- Component E:** Stop solution (10 mL)

### **OTHER MATERIALS REQUIRED (BUT NOT PROVIDED)**

**MMP-12:** Purified enzyme can be ordered from Sigma (Cat# M7442).

**96-well or 384-well microplate:** Black microplate provides better signal to noise ratio.

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

## **STANDARD OPERATION PROTOCOL**

*Note: For fluorometer calibration, please refer to Appendix II (recommended for first time users).*

### **1. Prepare MMP-2 containing biological samples**

- Collect synovial fluids or supernatant of cell culture media (e.g. stimulated fibroblast) and centrifuge for 5 min at 10,000X g, 4°C. Collect the supernatant and store at -70°C until use.
- Tissue samples should be homogenized in assay buffer (component D) containing 0.1% (v/v) Triton-X 100, and then centrifuged for 15 min at 10000x g at 4°C. Collect the supernatant and store at -70°C until use.

*Note 1: Triton-X 100 is not provided.*

*Note 2: MMPs are usually present as a mixture in cells or tissue and since some MMPs recognize and cleave the same peptide substrate, you may need to purify MMP-12 by immuno-affinity purification or other methods first before measuring its activity using our assay kit. According to our data, the FRET substrate in this kit can also be cleaved by MMP-3, but not MMP-1, 2, 7, 8, 9, 13, and 14.*

### **2. Activate pro-MMPs**

- Incubate the MMP containing-samples with APMA (component C) at a final concentration of 1 mM in the assay buffer (component D) for 15 min at 37°C. Activate MMP immediately before the experiment.

*Note 1: Keep the enzyme-containing sample on ice. Avoid vigorously vortexing the enzyme.*

*Prolonged storage will de-activate the enzyme.*

*Note 2: APMA belongs to organic mercury. Handle with care! Do not dump into sink!*

### **3. Prepare working solutions**

*Note: Warm all kit components until thawed to room temperature before starting the experiments.*

- MMP-12 substrate solution: Dilute MMP-12 substrate (component A) 1: 100 in assay buffer (component D).
- MMP-12 diluent: If you use purified MMP-12, dilute MMP-12 to appropriate concentration in assay buffer (component D).

*Note: Pro-MMP-12 needs to be activated before use, please refer to Step 2. Avoid vigorous vortexing of enzyme.*

### **4. Set up the enzymatic reaction**

- Add 50  $\mu$ L/well MMP-12 containing sample to a 96-well plate, or 20  $\mu$ L/well to a 384-well plate.
- Set up the following control:
  - Substrate control contains assay buffer (50  $\mu$ L/well for a 96-well plate or 20  $\mu$ L/well for a 384-well plate).

### **5. Initiate the enzymatic reaction**

- Add 50  $\mu$ L/well (96-well plate) or 20  $\mu$ L/well (384-well plate) of MMP-12 substrate solution to the sample and control wells. Mix the reagents by shaking the plate gently for 30 seconds.

- Measure fluorescence signal:

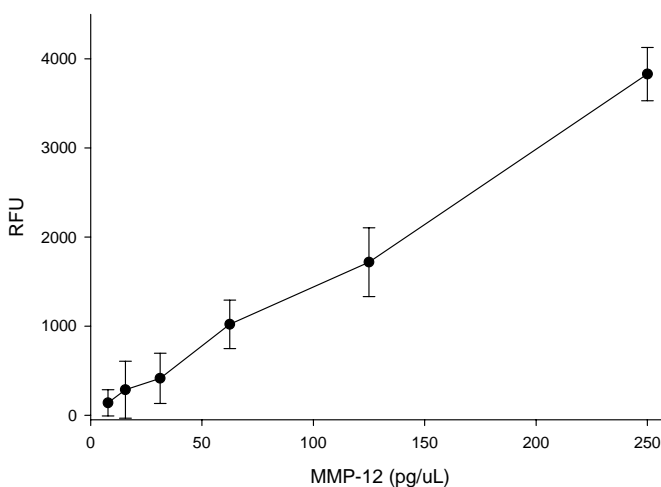
For kinetic reading: Immediately start measuring fluorescence intensity at Ex/Em=490 nm/520 nm continuously and record data every 5 minutes for 30 to 60 minutes.

For end-point reading: Incubate the reaction at room temperature for 30 to 60 minutes. Keep the plate away from direct light. (Optional) add 50  $\mu$ L/well (96-well plate) or 20  $\mu$ L/well (384-well plate) of stop solution (component E). Mix the reagents, then measure fluorescence intensity at Ex/Em=490 nm/520 nm.

- Data analysis: Refer to Appendix I.

## Appendix I Data Analysis

- The fluorescence reading from the substrate 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. To convert RFU to concentration of the product of enzymatic reaction, please refer to [Appendix II](#) for setting up the fluorescence reference standard.
  - 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. Determine the slope of the linear portion of the data plot.
  - A variety of data analyses can be done, e.g., determining inhibition %,  $EC_{50}$ ,  $IC_{50}$ ,  $K_m$ ,  $K_i$ , etc.
- For endpoint reading:
  - Plot data as RFU versus concentration of test compounds.
  - A variety of data analyses can be done, e.g., determining inhibition %,  $EC_{50}$ ,  $IC_{50}$ , etc.
- The sensitivity of this FRET-based assay was showed in **Figure 1**.



**Figure 1.** The sensitivity of EnzoLyte™ 520 MMP-12 assay kit.

The purified MMP-12 was first activated with 1 mM APMA and then serially diluted in assay buffer. The enzyme at each dilution was mixed with MMP-12 FRET substrate and then incubated at RT for 30 min. The endpoint fluorescence signal was recorded at  $Ex/Em=490\text{ nm}/520\text{ nm}$  with cut off 515 nm on FlexStation 384II (Molecular Device, CA). The assay is able to detect as low as 10 pg/μL of MMP-12. (mean±S.D., n=2).

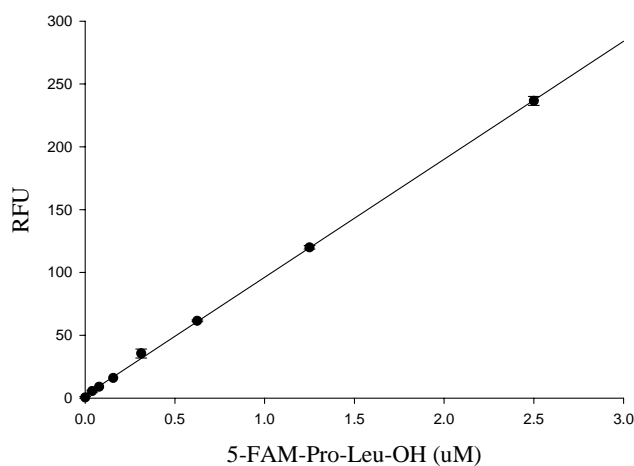
## Appendix II: Fluorometer Calibration

- **5-FAM fluorescence reference standard:** Dilute 1 mM 5-FAM-Pro-Leu-OH (component B) to 5  $\mu\text{M}$  in deionized water. Perform 1:2 serial dilutions to get concentrations of 2.5, 1.25, 0.625, 0.3125, 0.156, 0.078, and 0  $\mu\text{M}$ . Add 50  $\mu\text{L}$ /well (96-well plate) or 20  $\mu\text{L}$ /well (384-well plate) of the serially diluted 5-FAM-Pro-Leu-OH from 5  $\mu\text{M}$  to 0 nM.
- Add 50  $\mu\text{L}$ /well (96-well plate) or 20  $\mu\text{L}$ /well (384-well plate) of MMP-12 substrate solution (refer to Step 3 of the Standard Operation Protocol for preparing substrate solution).

*Note:* The MMP-12 substrate solution should be added to the 5-FAM reference standard to correct for the fluorescence inner filter effect.

- (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 the reference standard wells to obtain a better comparison.
- Plot 5-FAM fluorescence reference standard as RFU (relative fluorescence unit) versus concentration as **Figure 2**.

*Note:* The final concentrations of the 5-FAM-Pro-Leu-OH reference standard solutions are 2.5, 1.25, 0.625, 0.3125, 0.156, 0.078, 0.039, and 0  $\mu\text{M}$ . This reference standard curve is used to calibrate for the variation of different instruments and for different batches of experiments. It is also an indicator of the amount of MMP-12 enzymatic reaction final product.



**Figure 2.** The 5-FAM-Pro-Leu-OH reference standard curve.

5-FAM-Pro-Leu-OH was serially diluted in assay buffer according to the protocol. The fluorescence was monitored by a fluorescence microplate reader (Bio-Tek FLx800) with a filter set of Excitation/Emission=485 $\pm$ 20 nm/528 $\pm$ 20 nm. (mean $\pm$ S.D., n=2).

## References

1. J. F. Woessner, Jr. and C. J. Taplin, *J.Biol.Chem.* 263, 16918-16925 (1988).
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3. R. D. Hautamaki, D. K. Kobayashi, R. M. Senior, S. D. Shapiro, *Science* 277, 2002-2004 (1997).
4. Z. Dong, R. Kumar, X. Yang, I. J. Fidler, *Cell* 88, 801-810 (1997).
5. L. Stryer, *Annu.Rev.Biochem.* 47, 819-846 (1978).