Amplite™ Universal Fluorimetric MMP Activity Assay Kit
Green Fluorescence

Ordering Information:
Product number: 13510 (100 assays)

Instrument Platform:
Fluorescence microplate readers

Storage Conditions:
Keep in -20°C avoid light. *Components C can be stored at 4 °C for convenience.*

Introduction

The matrix metalloproteinases (MMPs) constitute a family of zinc-dependent endopeptidases that function within the extracellular matrix. These enzymes are responsible for the breakdown of connective tissues and are important in bone remodeling, the menstrual cycle and repair of tissue damage. While the exact contribution of MMPs to certain pathological processes is difficult to assess, MMPs appear to play a key role in the development of arthritis as well as in the invasion and metastasis of cancer. MMPs tend to have multiple substrates, with most family members having the ability to degrade different types of collagen along with elastin, gelatin and fibronectin. This kit is designed to check the general activity of a MMP enzyme. It can also be used to screening MMP inhibitors when a purified MMP enzyme is used.

Our Amplite™ Universal Fluorimetric MMP Activity Assay Kit uses a TF2™/TQ2™ fluorescence resonance energy transfer (FRET) peptide as a MMP substrate. In the intact FRET peptide, the fluorescence of TF2™ is quenched by TQ2™. Upon cleavage into two separate fragments by MMPs, the fluorescence of TF2™ is recovered. With excellent fluorescence quantum yield and longer wavelength, TF2™ shows less interference from autofluorescence of test compounds and cellular components and is much more sensitive than an EDANS/Dabcyl FRET substrate. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation with no separation steps required. Its signal can be easily read by fluorescence microplate reader with Ex/Em = 490 nm/520 nm.

Kit Features and Benefits

Convenient Format:	Kit includes all the key assay components.
Optimized Performance:	Optimized conditions for the detection of generic MMP protease activity.
Continuous:	Easily adapted to automation with no separation required.
Convenient:	Formulated to have minimal hands-on time. No wash is required.
Non-Radioactive:	No special requirements for waste treatment.

Kit Components

Components	Amount
Component A: MMP Green™ substrate	1 vial (60 µL), protect from light
Component B: APMA, 4-aminophenylmercuric acetate	1 vial (20 µL, 1M)
Component C: Assay buffer	1 bottle (20 mL)

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Assay Protocol for one 96-well plate

Brief Summary

Prepare MMP Green™ substrate solution (50 µL) → Add appropriate controls, or test samples (50 µL), and pre-incubate for 10-15 min → Incubate for 0 min (for kinetic reading) or 30 min-1 h (for end point reading) → Read fluorescence at Ex 490 nm/Em 520nm

Thaw all the kit components to room temperature before starting your experiment.

1. Prepare MMPs containing biological samples as desired.

2. Activate pro-MMPs:

2.1 Make 1mM APMA working solution: Dilute APMA (Component B) with assay buffer (Component C) at 1:1000 to 1 mM.
Note: APMA belongs to organic mercury. Handle with care! Dispose it according to local regulations.

2.2 Incubate the MMPs with APMA: Incubate the MMP containing-samples or purified MMPs with 1 mM APMA (from step 2.1). Refer to Appendix I for incubation time. Activate MMP immediately before the experiment.
Note 1: Keep enzyme-containing samples on ice. Avoid vigorously vortexing the enzyme. Prolonged storage of the activated enzyme will deactivate the enzyme; Note 2: For zymogen activation, it is preferably activated at higher protein concentration. After activation, you may dilute the enzyme further.

3. Prepare working solutions:

3.1 Make MMP Green™ substrate solution: Dilute MMP Green™ substrate (Component A) with assay buffer (Component C) at 1:100.

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

Components	Volume
MMP Green™ substrate (Component A)	50 µL
Assay buffer (Component C)	5 mL
Total volume	5 mL

3.2 Make MMP dilution: Dilute MMPs to an appropriate concentration in assay buffer (Component C) if you use purified MMP.
Note: Pro-MMP needs to be activated before use (see Step 2.2). Avoid vigorous vortexing of the enzyme.

3.3 Make inhibitors and compounds dilution: Make an appropriate concentration of known MMPs inhibitors and test compounds dilutions as desired if you are screening MMPs inhibitors.

4. Set up the enzymatic reaction in a 96-well microplate according to Table 2 and Table 3.

Table 2. Layout of the appropriate controls (as desired) and test samples in a 96-well microplate:

SC	SC										
IC	IC										
VC	VC										
TC	TC										
TS	TS										
….	….										
….	….										

Note: SC= substrate control, IC= Inhibitor control, VC=Vehecle control, TC= Test compound control, TS=test samples.

Table 3. Reagent composition for each well:

Substrate Control	Inhibitor Control	Vehicle Control	Test Compound Control*	Test Sample
Assay buffer	MMP dilution and known MMPs inhibitor:	MMP dilution and vehicle used to deliver test compound:	MMP-containing assay buffer and test compound:	MMP dilution with test compound:
Total volume: 50 µL	50 µL	50 µL	50 µL	50 µL

Note 1: *Some strongly auto- fluorescent test compounds may give false results;
Note 2: Make the total volume of all the controls to 50 µL for 96-well plate or 20 µL for 384-well plate *by assay buffer(Component C).*

5. Run the enzyme reaction.

4.1 Pre-incubate the plate at the desired temperature for the enzyme reaction (e.g. 25°C or 37°C) for 10-15 min if you are screening MMPs inhibitors.

4.2 Add 50 µL (20 µL for 384-well plate) MMP Green™ substrate solution (from step 3.1) to the sample and control wells into the assay plate. Mix the reagents well.

4.3 Monitor the fluorescence intensity with 490 nm excitation and 520 nm emission by using a fluorescence plate reader.

For kinetic reading: Immediately start measuring fluorescence intensity 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, avoiding light if possible. Mix the reagents well, and then measure the fluorescence intensity.

6. Data analysis:

The fluorescence in the substrate control well is used as a control, and is subtracted from the values for other wells with the enzyme reactions. Plot data as RFU versus concentration of test compounds or enzyme concentration **(Figure 1).** In addition, a variety of data analyses can also be determined, e.g., determining inhibition %, EC_{50}, IC_{50}, etc.

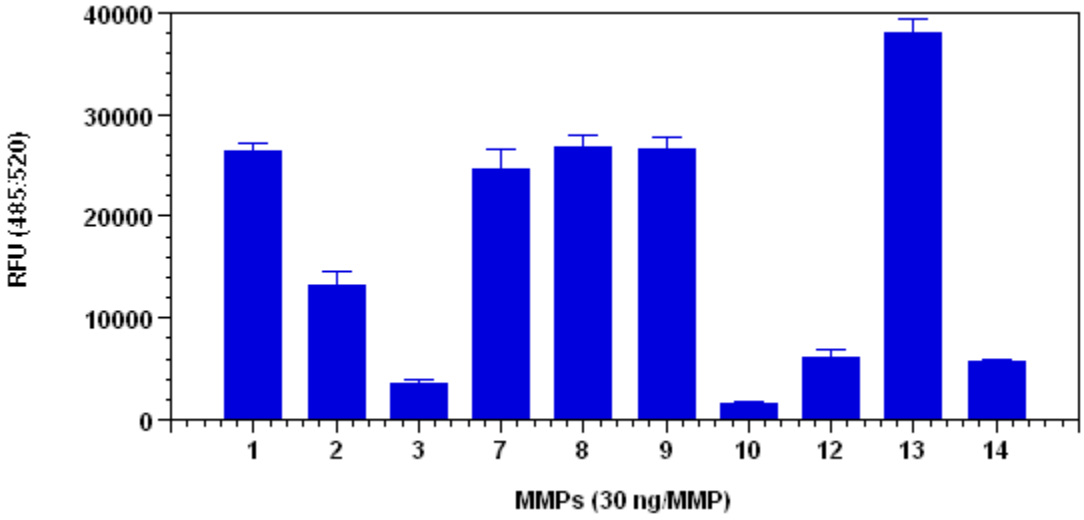

Figure 1. Detect the activity of MMPs using Amplite™ Universal Fluorimetric MMP Activity Assay Kit. The APMA-activated MMPs, 30 ng each, were mixed with MMP Green™ substrate. The fluorescence signal was monitored at 1 hr after the start of the reaction by using a BMG LabTech NOVOStar microplate reader with a filter set of Ex/Em=485 nm/520 nm. The reading from all wells was subtracted with the reading from substrate control, which contains MMP Green™ substrate but no MMPs. Although different MMPs showed different cleavage rate on this MMP substrate, the MMP Green™ substrate can detect the activity of sub-nanogram of all MMPs (n=3).

Appendix I: 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 1 h.
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.

References:

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