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Amplite™ Universal Fluorimetric MMP Activity Assay Kit
Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 13511 (100 assays)	Store at -20 °C and keep from light Component C can be stored at 4 °C for convenience	Fluorescence microplate readers

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.

This Amplite™ Universal Fluorimetric MMP Activity Assay Kit uses a Tide Fluor™ 3 (TF3)/Tide Quencher™ 3 (TQ3) fluorescence resonance energy transfer (FRET) peptide as a MMP substrate. In the intact FRET peptide, the fluorescence of TF3 is quenched by TQ3. Upon cleavage into two separate fragments by MMPs, the fluorescence of TF3 is recovered. This kit is designed to check the general activity of a MMP enzyme. It can also be used to screen MMP inhibitors when a purified MMP enzyme is used.

With excellent fluorescence quantum yield and longer wavelength, TF3 shows less interference from autofluorescence of test compounds and cellular components and is much more sensitive than an EDANS/Dabcyl FRET substrate. Its signal can be easily read by a fluorescence microplate reader at Ex/Em = 540/590 nm. The pH-independent fluorescence of TF3 makes the assay reading available for the whole physiological pH range. The high photostability of TF3 might make the TF3/TQ3 FRET peptide a useful imaging probe. Many labs have used this kit for the high throughput screening of MMP inhibitors as potential anticancer drug candidates. This assay might be also used for monitoring cancer cells.

Kit Key Features

Convenient Format:	Include all the key assay components.
Optimized Performance:	Optimal conditions for the detection of generic protease activity.
Continuous:	Easily adapted to automation without a separation step.
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 Red™ Substrate	1 vial (60 µL), protected from light
Component B: APMA, 4-Aminophenylmercuric Acetate	1 vial (20 µL, 1 M)
Component C: Assay Buffer	1 bottle (20 mL)

Assay Protocol for One 96-Well Plate

Brief Summary

Add appropriate controls, or test samples (50 µL) → Pre-incubate for 10-15 mintues → Add 50 µL of MMP Red™ substrate solution → Incubate for 0 minute (for kinetic reading) or 30 minutes-1 hour (for end point reading) → Monitor fluorescence intensity at Ex/Em = 540/590 nm

Note: Thaw all the kit components at room temperature before starting the experiment.

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Technical Support: support@aatbio.com; Tel: 408-733-1055

1. Prepare MMPs containing biological samples as desired.

2. Activate pro-MMPs:

2.1 Make 2 mM APMA working solution (2X): Dilute 1 M APMA (Component B) with Assay Buffer (Component C) at 1:500 to 2 mM APMA working solution (2X).
Note: APMA belongs to organic mercury. Handle with care! Dispose it according to local regulations.

2.2 Incubate MMPs with APMA: Incubate the MMP containing-samples or purified MMPs with equal volume of 2 mM APMA working solution (2X, 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 enzyme activation, it is preferably activated at higher protein concentration. After activation, you may further dilute the enzyme.

3. Prepare working solutions:

3.1 Make MMP Red™ substrate working solution: Dilute MMP Red™ Substrate solution (Component A) with Assay Buffer (Component C) at 1:100 as shown in **Table 1**.

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

Components	**Volume**
MMP Red™ 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 with Assay Buffer (Component C) if purified MMP is used.
Note: Pro-MMP needs to be activated before use (see Step 2.2). Avoid vigorously vortexing the enzyme.

3.3 Make inhibitors and compounds dilution: Make dilutions of known MMPs inhibitors and test compounds 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=Vehicle 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	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 fluorescent test compounds may result in false-positive results.*
Note 2: Make the total volume of all the controls to 50 µL for a 96-well plate or 20 µL for a 384-well plate by using Assay Buffer (Component C).

5. Run the enzyme reaction:

5. 1 Pre-incubate the plate at a desired temperature for the enzyme reaction (e.g. 25 °C or 37 °C) for 10-15 min if you are screening MMPs inhibitors.

5. 2 Add 50 µL(96-well) or 20 µL (384-well) of MMP Red™ substrate working solution (from Step 3.1) to the sample and control wells of the assay plate. Mix the reagents well.

5. 3 Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em = 540 /590 nm.
For kinetic reading: Immediately start measuring fluorescence intensity and continuously 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, kept from light if possible. Mix the reagents well, and then measure the fluorescence intensity.

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 **(**as shown in Figure 1**).** In addition, a variety of data analyses can also be determined, e.g., determining inhibition %, EC_{50}, IC_{50}, etc.

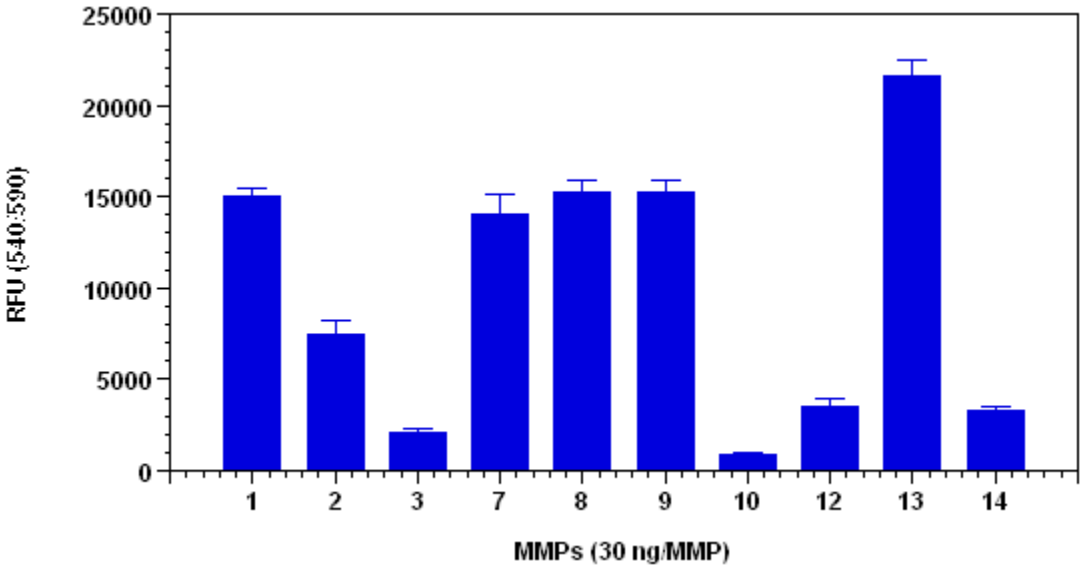

Figure 1. Detect the activity of MMPs using Amplite™ Universal Fluorimetric MMP Activity Assay Kit. The APMA-activated MMPs, 30 ng each, were mixed with MMP Red™ substrate. The fluorescence signal was monitored one hour after the start of the reaction by using a NOVOStar microplate reader (BMG Labtech) with a filter set of Ex/Em = 540/590 nm. The reading from all wells was subtracted with the reading from substrate control, which contains MMP Red™ substrate but no MMPs. Although different MMPs showed different cleavage rate on this MMP substrate, the MMP Red™ 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 hr.
MMP-2 (gelatinase)	1 mM APMA (diluted component C) at 37 °C for 1 hr.
MMP-3 (stromelysin)	1 mM APMA (diluted component C) at 37 °C for 24 hr.
MMP-7 (matrilysin, PUMP-1)	1 mM APMA (diluted component C) at 37 °C for 20 min-1 hr.
MMP-8 (neutrophil collagenase)	1 mM APMA (diluted component C) at 37 °C for 1 hr.
MMP-9 (92 kDa gelatinase)	1 mM APMA (diluted component C) at 37 °C for 2 hr.
MMP-10 (stromelysin 2)	1 mM APMA (diluted component C) at 37 °C for 24 hr.
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 hr.
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 hr.

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