Amplite™ MMP-3 Activity Assay Kit

Green Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 13512 (100 assays)	Store at -20 °C and keep from light Component C can be stored at 4 °C for convenience	Fluorescence microplate readers

Introduction

MMP-3 (Matrix Metalloproteinase-3), also known as stromelysin-1, is involved in wound repair, atherosclerosis and tumors. Our Amplite™ Fluorimetric MMP-3 Activity Assay Kit uses a Tide Fluor™ 2 (TF2)/Tide Quencher™ 2 (TQ2) fluorescence resonance energy transfer (FRET) peptide as the MMP-3 activity indicator. In the intact FRET peptide, the fluorescence of TF2 is quenched by TQ2. Upon cleavage into two separate fragments by MMP-3, the green fluorescence of TF2 is recovered.

This kit is designed to monitor the activity of an MMP-3 enzyme. The poptide sequence used in the kit is more selective for MMP-3 hydrolysis than other MMP enzymes. It can also be used to screen MMP-3 inhibitors when a purified MMP-3 enzyme is used. 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. Its signal can be easily read by a fluorescence microplate reader at Ex/Em = 490/525 nm. The perfect excitation of TF2 at 488 nm makes the assay readily readable with almost all the common fluorescence instruments equipped with Argon laser and FITC filter set.

Kit Key Features

Optimized Performance:	Optimized conditions for the detection of MMP-3 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-3 Green™ 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 minutes → Add MMP-3 Green™ substrate solution (50 µL) → Incubate for 0 min (for kinetic reading) or 30 minutes - 1 hour (for end point reading) → Monitor fluorescence intensity at Ex/Em = 490/525 nm

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

1. Prepare MMP-3 containing biological samples as desired.

2. Activate pro-MMP-3:

2.1 Make 2 mM APMA working solution (2X): Dilute 1 M APMA (Component B) with Assay Buffer (Component C) at 1:500 to get a 1 mM APMA working solution (2X)

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Note: APMA belongs to organic mercury. Handle with care! Dispose it according to local regulations.

2.2 Incubate the MMP-3 with 1 mM APMA: Incubate the MMP-3 containing-samples or purified MMP-3 with equal volume of 2 mM APMA working solution (2X, from Step 2.1) at 37 °C for 24 hours. Activate MMP-3 immediately before the experiment.
Note1: 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-3 Green™ substrate working solution: Dilute MMP-3 Green™ Substrate (Component A) with Assay Buffer (Component C) at 1:100 as shown in **Table 1**.

Table 1 MMP-3 Green™ substrate working solution for one 96-well plate (100 assays)

Components	**Volume**
MMP-3 Green™ Substrate (Component A)	50 µL
Assay Buffer (Component C)	5 mL
Total volume	5 mL

3.2 Make MMP-3 dilution: Dilute MMP-3 to an appropriate concentration in Assay Buffer (Component C) if purified MMP-3 is used.
Note: Pro-MMP-3 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 MMP-3 inhibitors and test compounds dilutions as desired if you are screening MMP-3 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-3 dilution and known MMP-3 inhibitor	MMP-3 dilution and vehicle used to deliver test compound	MMP-3 containing assay buffer and test compound	MMP-3 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 minutes if you are screening MMP-3 inhibitors.

5.2 Add 50 µL (96-well) or 20 µL (384-well) of MMP-3 Green™ 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 = 490/525 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 analysis can also be determined, e.g., determining inhibition %, EC_{50}, IC_{50}, etc.

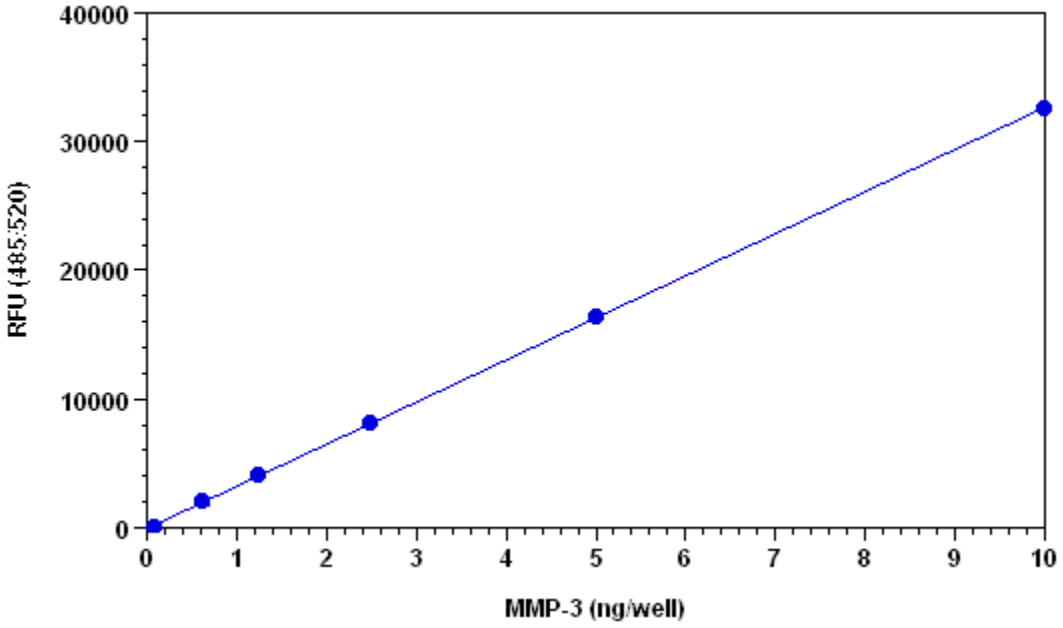

Figure 1. Dose response of MMP-3 enzyme activity. The APMA-activated MMP-3 was serially diluted in assay buffer, and then mixed with MMP-3 Green™ substrate. The fluorescence signal was monitored one hour after the start of the reaction by using a NOVOStar microplate reader (BMG Labtech) with a filter set of Ex/Em = 485/525 nm. The reading from all wells was subtracted with the reading from substrate control, which contains MMP-3 Green™ substrate but no MMP-3. As low as 1 ng/well of MMP-3 was detected (n=3). *Note: MMP-3 from different sources varies in its endogenous activity.*

References

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