Amplite™ Universal Fluorimetric MMP Activity Assay Kit *Green Fluorescence*

Catalog number: 13510
Unit size: 100 Tests

Component	Storage	Amount
Component A: MMP Green™ Substrate	Freeze (<-15 °C), Minimize light exposure	1 vial (60 µL)
Component B: APMA, 4-Aminophenylmercuric Acetate	Freeze (<-15 °C), Minimize light exposure	1 vial (20 µL, 1 M)
Component C: Assay Buffer	Freeze (<-15 °C)	1 bottle (20 mL)

OVERVIEW

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. It is quite difficult to find a substrate that is selective to a single MMP enzyme. 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. We also offer a few MMP enzyme of high activity.

AT A GLANCE

Protocol summary

1. Add appropriate controls or test samples (50 µL)
2. Pre-incubate for 10 - 15 minutes
3. Add MMP Green™ substrate working solution (50 µL)
4. Skip incubation step for kinetic reading or incubate for 30 to 60 minutes for end point reading
5. Monitor fluorescence intensity at Ex/Em = 490/525 nm

Important Thaw all the kit components at room temperature before starting the experiment. Prepare MMPs containing biological samples as desired.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490 nm
Emission:	525 nm
Cutoff:	515 nm
Recommended plate:	Solid black

PREPARATION OF WORKING SOLUTION

1. MMP Green™ Substrate working solution:
Add 50 µL of MMP Green™ Substrate (Component A) into 5 mL of Assay Buffer (Component C) to make a total volume of 5.05 mL of MMP Green™ substrate working solution.

2. MMP dilution:
Dilute MMPs to an appropriate concentration in Assay Buffer (Component C) if purified MMP is used.

Note MMP needs to be activated before use. Avoid vortexing the enzyme vigorously.

3. Inhibitors and compounds dilution:
Make dilutions of known MMPs inhibitors and test compounds as desired if you are screening MMPs inhibitors.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of the appropriate controls (as desired) and test samples in a 96-well microplate. SC= Substrate Control, IC= Inhibitor Control, VC=Vehicle Control, TC= Test Compound Control, TS=Test Samples.

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

Table 2. Reagent composition for each well. Some strongly fluorescent test compounds may result in false-positive results. 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).

Well	Volume	Reagent
SC	50 µL	Assay Buffer
IC	50 µL	MMP dilutions and known MMPs inhibitors
VC	50 µL	MMP dilution and vehicle used to deliver test compound
TC	50 µL	MMP-containing assay buffer and test compound
TS	50 µL	MMP dilution with test compound

Table 3. Protocols for 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 h
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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1. Prepare MMPs containing biological samples as desired.

2. To activate pro-MMPs, first dilute 1 M APMA (Component B) with Assay Buffer (Component C) at 1:500 to get 2 mM APMA working solution (2X).

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

   Next, incubate the MMP containing-samples or purified MMPs with equal volume of 2 mM APMA working solution (2X). Refer to Table 3 for incubation time. Activate MMPs immediately before the experiment.

   Note Keep enzyme-containing samples on ice. Avoid vigorously vortexing the enzyme. Prolonged storage of the activated enzyme will deactivate the enzyme. For enzyme activation, it is preferably activated at higher protein concentration. After activation, you may further dilute the enzyme.

3. Prepare controls and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 20 µL of reagent per well instead of 50 µL.

4. 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. Add 50 µL (96-well) or 20 µL (384-well) of MMP Green™ substrate working solution to the sample and control wells of the assay plate. Mix the reagents well.

6. 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.

EXAMPLE DATA ANALYSIS AND FIGURES

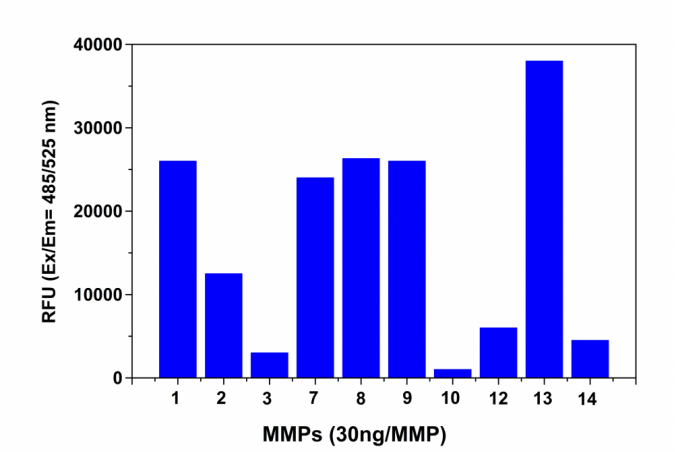

Figure 1. Detection of MMPs activity using Amplite™ Universal Fluorimetric MMP Activity Assay Kit. The APMA-activated MMPs, 30 ng each, were mixed with MMP Green™ substrate. The fluorescence signal was monitored one hour after starting the the reaction by using a NOVOStar microplate reader (BMG Labtech) with a filter set of Ex/Em = 490/525 nm. The reading from all wells was subtracted with the reading from substrate control, which contains MMP Green™ substrate but no MMPs. The MMP Green™ substrate can detect the activity of sub-nanogram of all MMPs (n=3).

DISCLAIMER