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

Catalog number: 13511
Unit size: 100 Tests

Component	Storage	Amount
Component A: MMP Red™ 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. Prepare appropriate controls or test samples (50 µL)
2. Pre-incubate for 10 -15 minutes
3. Add MMP Red™ Substrate working solution (50 µL)
4. Skip incubation for kinetic reading or incubate 30 minutes - 1 hour for end point reading
5. Monitor fluorescence intensity at Ex/Em = 540/590 nm (Cutoff = 570 nm)

Important Thaw all the kit Components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Solid black

PREPARATION OF WORKING SOLUTION

1. APMA working solution (2mM, 2X):
Dilute 1 M APMA (Component B) with Assay Buffer (Component C) at 1:500 to make 2 mM, 2X APMA working solution .

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

2. MMP Red™ Substrate working solution:
Add 50 µL of MMP Red™ Substrate (Component A) to 5 mL of Assay Buffer (Component C) and mix well to make MMP Red™ Substrate working solution.

Note MMP Red™ Substrate working solution is enough for one 96-well plate (100 assays).

3. MMP dilution:
Dilulte MMPs to an appropriate concentration with Assay Buffer (Component C) if purified MMP is used.

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

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

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. 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.

Table 2. Layout of the samples in a solid black 96-well microplate. SC=Substrate Control, IC=Inhibitor Control, VC=Vehicle Control, TC=Test Compound Control, TS=Test Samples.

SC	SC	...	...
IC	IC	...	...

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VC	VC		
TC	TC		
TS	TS		
...	...		

Table 3. Reagent composition for each well. Some strongly fluorescent test compounds may result in false-positive results.

Well	Volume	Reagent
SC	50 µL	Assay Buffer
IC	50 µL	MMP dilution and known MMPs inhibitor
VC	50 µL	MMP dilution and vehicle used to deliver test compound
TC	50 µL	Assay Buffer and test compound
TS	50 µL	MMP dilution with test compound

1. Prepare MMPs containing biological samples as desired.

2. Incubate the MMP containing-samples or purified MMPs with equal volume of 2 mM APMA working solution (2X). Refer to Table 1 for incubation time. Activate MMP 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.

   Note For enzyme activation, it is preferably activated at higher protein concentration. After activation, you may further dilute the enzyme.

3. Prepare Subtrate Control (SC), Inhibitor Control (IC), Vehicle Control (VC), Test Compound Control (TC) and Test Samples (TS) according to the layout provided in Table 2 and Table 3.

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/well (96-well plate) or 20 µL/well (384-well plate) of MMP Red™ 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 = 540/590 nm (Cutoff = 570 nm).

   For kinetic reading: Immediately start measuring fluorescence intensity continuously and record data every 5 minutes for 30 minutes.

   For end-point reading: Incubate the reaction at a desired temperature for 30 to 60 minutes, protected from light. Mix the reagents well, and then measure the fluorescence intensity.

EXAMPLE DATA ANALYSIS AND FIGURES

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.

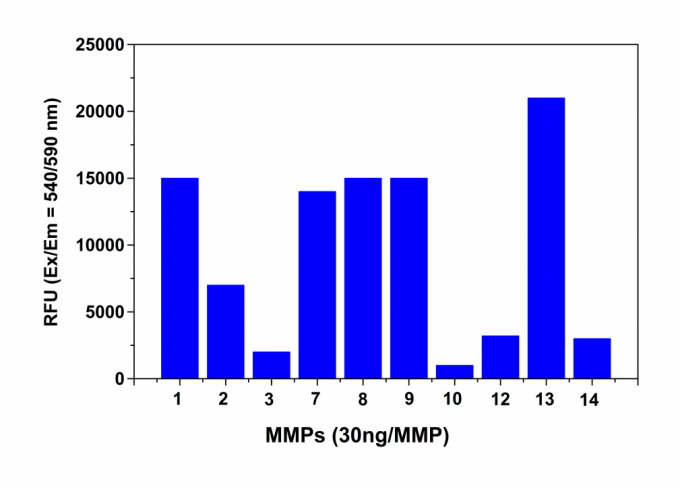

Figure 1. Detection of MMPs activity using Amplite™ Universal Fluorimetric MMP Activity Assay Kit. The fluorescence signal was monitored one hour after the start of the reaction. The reading from all wells was subtracted with the reading from substrate control, which contains MMP Red™ substrate but no MMPs. The MMP Red™ substrate can detect the activity of sub-nanogram of all MMPs (n=3).

DISCLAIMER