Amplite™ MMP-3 Activity Assay Kit *Green Fluorescence*

Catalog number: 13512
Unit size: 100 Tests

Component	Storage	Amount
Component A: MMP-3 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 FRET substrate is designed to have a relative high selectivity to MMP-3. It is used to monitor the MMP-3 activity. It can also be used to screening MMP-3 inhibitors when a purified MMP-3 enzyme is used. This FRET substrate is based on our TF2/TQ2 FRET pair. Upon MMP-3 hydrolysis the fluorescence of MMP-3 Green™ FRET peptide substrate is increased since the TF2/TQ2 FRET pair is separated. The fluorescence increase is proportional to the MMP-3 enzyme activities.

AT A GLANCE

Protocol summary

1. Add appropriate controls, or test samples (50 µL)
2. Pre-incubate for 10 - 15 minutes
3. Add MMP-3 Green™ substrate working solution (50 µL)
4. Skip incubation for kinetic reading or incubate 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 MMP-3 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-3 Green™ Substrate working solution:*
Add 50 µL of MMP-3 Green™ Substrate (Component A) into 5 mL of Assay Buffer (Component C) to make a total volume of 5.05 mL.

2. *MMP-3 dilution:*
Dilute MMP-3 to an appropriate concentration in Assay Buffer (Component C) if purified MMP-3 is used.

Note MMP-3 needs to be activated before use. Avoid vigorous vortexing of the enzyme.

3. *Inhibitors and compounds dilution:*
Make an appropriate concentration of known MMP-3 inhibitors and test compounds dilutions as desired if screening MMP-3 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.

Note Some strongly fluorescent test compounds may result in false-positive results.

Well	Volume	Reagent
SC	50 µL	Assay Buffer (Component C)
IC	50 µL	MMP-3 dilution and known MMP-3 inhibitor
VC	50 µL	MMP-3 dilution and vehicle used to deliver test compound
TC	50 µL	MMP-3 containing assay buffer and test compound
TS	50 µL	MMP-3 dilution with test compound

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

2. To activate pro-MMP-3, first dilute 1M APMA (Component B) with Assay Buffer (Component C) at 1:500 to get a 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-3 containing-samples or purified MMP-3 with equal volume of 2 mM APMA working solution (2X) at 37 °C for 24 hours. Activate MMP-3 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.

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4. 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. Add 50 µL (96-well) or 20 µL (384-well) of MMP-3 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

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate MMP-3 samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

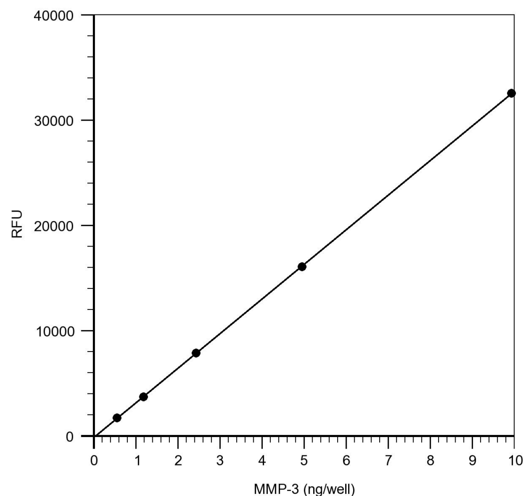

Figure 1. Dose response of MMP-3 enzyme activity was measured with Amplite™ MMP-3 Activity Assay Kit using a NOVOStar microplate reader (BMG Labtech).

DISCLAIMER

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