MMP-3 Green™ substrate solution

Catalog number: 13527
Unit size: 100 Tests

Component	Storage	Amount
MMP-3 Green™ substrate Solution	Freeze (< -15 °C), Minimize light exposure	100 tests

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 the solution at room temperature before starting the experiment. Prepare MMP-3 containing biological samples as desired.

KEY PARAMETERS

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 Solution into 5 mL of buffer of your choice to make a total volume of 5.05 mL. **Note:** Tris buffer can be used for the assay.

2. MMP-3 dilutions
Dilute MMP-3 to an appropriate concentration in buffer of your choice 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

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

2. Activate pro-MMP-3 as per protocol. **Note:** 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.

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 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. **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.

Well	Volume	Reagent
SC	50 µL	Buffer of your choice
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 buffer and test compound
TS	50 µL	MMP-3 dilution with test compound

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