MMP-3 Green™ substrate

Catalog number: 13528
Unit size: 1 mg

Component	Storage	Amount
MMP-3 Green™ substrate	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)

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 powder 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 STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

MMP-3 Green™ Substrate stock solution
Dissolve MMP-3 Green™ Substrate in DMSO to make 1-2 mM stock solution. **Note:** Store MMP-3 Green™ Substrate stock solution in single use aliquots.

PREPARATION OF WORKING SOLUTION

1. MMP-3 Green™ Substrate working solution
Dilute MMP-3 Green™ Substrate stock solution into buffer of your choice to achieve 5 to 25 µM concentration of working solution. **Note:** Tris buffer can be used for the assay. **Note:** The appropriate concentration should be optimised based on application.

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

EXAMPLE DATA ANALYSIS AND FIGURES

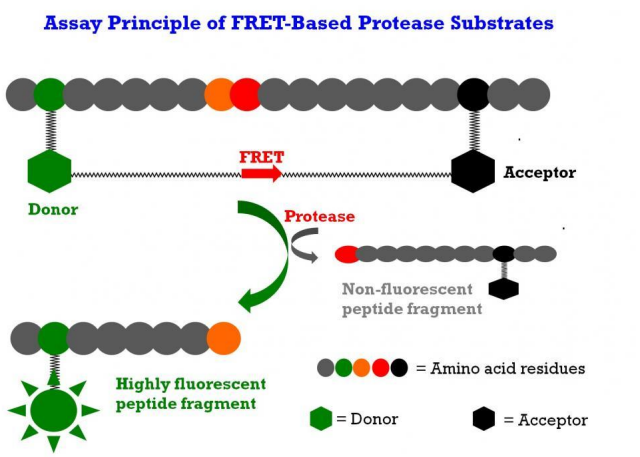

Figure 1.
The internally quenched FRET peptide substrate is digested by a protease to generate the highly fluorescent peptide fragment. The fluorescence increase is proportional to the protease activity.

DISCLAIMER

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