MMP Green™ substrate

Catalog number: 13519, 13520
Unit size: 100 Tests, 1 mg

Component	Storage	Amount (Cat No. 13519)	Amount (Cat No. 13520)
MMP Green™ Substrate	Freeze (< -15 °C), Minimize light exposure	100 tests	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 monitor the general activity of a MMP enzyme. It can also be used to screening MMP inhibitors when a purified MMP enzyme is used. This FRET substrate is based on our TF2/TQ2 FRET pair. Upon MMP hydrolysis the fluorescence of MMP Green™ FRET peptide substrate is increased since the TF2/TQ2 FRET pair is separated. The fluorescence increase is proportional to the MMP enzyme activities.

AT A GLANCE

Protocol summary

1. Add appropriate controls or test samples with or without APMA (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 the reagents includig the substrate at room temperature before starting the experiment. Prepare MMPs 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 Green™ Substrate stock solution
For Cat# 13520, prepare 1- 10 mg/mL stock solution by adding appropriate amount of DMSO to MMP Green™ substrate vial.

Note Store stock solution in single used aliquots and protect from light.

PREPARATION OF WORKING SOLUTION

MMP Green™ Substrate working solution

For Cat# 13520, prepare 20-100 µg/mL MMP Green™ Substrate working solution in buffer of your choice. For Cat# 13519, Add 50 µL of MMP Green™ solution (vial provided) into 5 mL of buffer of your choice.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. 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

1. Prepare MMPs containing biological samples as desired.

2. To activate pro-MMPs, add 2 mM APMA working solution (2X) to the plate.

   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 1 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) with 50 µL per well. 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 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

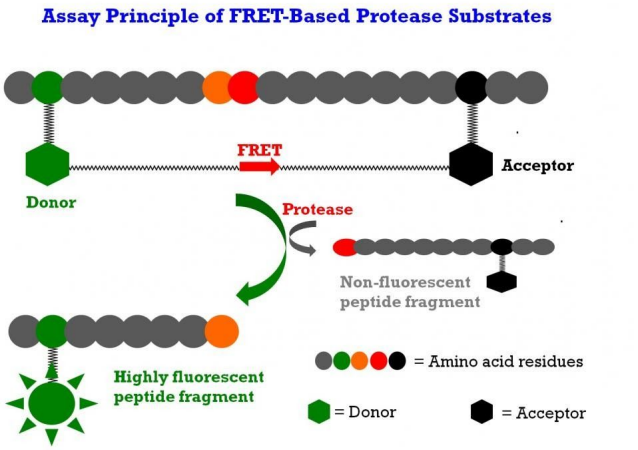

Figure 1.
The internally quenched FRET peptide substrate is digested by a protease to generate the highly fluorescent peptide fragment. The fluorescence increase is proportional to the protease activity.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.