CREATIVE BIOMART®
Assay Kit

MMP-3 Activity Assay Kit

Product Information

Common Name

MMP-3

Cat.No. Kit-0589

Product Overview

This kit is designed to monitor the activity of an MMP-3 enzyme. The poptide sequence used in the kit is more selective for MMP-3 hydrolysis than other MMP enzymes. It can also be used to screen MMP-3 inhibitors when a purified MMP-3 enzyme is used. With excellent fluorescence quantum yield and longer wavelength, TF2 shows less interference from autofluorescence of test compounds and cellular components and is much more sensitive than an EDANS/Dabcyl FRET substrate. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format. Its signal can be easily read by a fluorescence microplate reader at Ex/Em = 490/525 nm. The perfect excitation of TF2 at 488 nm makes the assay readily readable with almost all the common fluorescence instruments equipped with Argon laser and FITC filter set.

Description

MMP-3 (Matrix Metalloproteinase-3), also known as stromelysin-1, is involved in wound repair, atherosclerosis and tumors. Our Fluorimetric MMP-3 Activity Assay Kit uses a Tide Fluor 2 (TF2)/Tide Quencher 2 (TQ2) fluorescence resonance energy transfer (FRET) peptide as the MMP-3 activity indicator. In the intact FRET peptide, the fluorescence of TF2 is quenched by TQ2. Upon cleavage into two separate fragments by MMP-3, the green fluorescence of TF2 is recovered.

Storage

Store at -20 °C and keep from light. Component C can be stored at 4 °C for convenience

Size

100 assays

Kit Components

Component A: MMP-3 Green Substrate 1 vial (60 uL), protected from light

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Component B: APMA, 4-Aminophenylmercuric Acetate 1 vial (20 uL, 1 M)
Component C: Assay Buffer 1 bottle (20 mL)

Features & Benefits

Optimized Performance: Optimized conditions for the detection of MMP-3 protease activity.
Continuous: Easily adapted to automation without a separation step.
Convenient: Formulated to have minimal hands-on time. No wash is required.
Non-Radioactive: No special requirements for waste treatment.

Assay Protocol

Note: Thaw all the kit components at room temperature before starting the experiment.
1. Prepare MMP-3 containing biological samples as desired.
2. Activate pro-MMP-3:
2.1 Make 2 mM APMA working solution (2X): Dilute 1 M APMA (Component B) with Assay Buffer (Component C) at 1:500 to get a 1 mM APMA working solution (2X).
Note: APMA belongs to organic mercury. Handle with care! Dispose it according to local regulations.
2.2 Incubate the MMP-3 with 1 mM APMA: Incubate the MMP-3 containing-samples or purified MMP-3 with equal volume of 2 mM APMA working solution (2X, from Step 2.1) at 37 °C for 24 hours. Activate MMP- 3 immediately before the experiment.
Note1: Keep enzyme-containing samples on ice. Avoid vigorously vortexing the enzyme. Prolonged storage of the activated enzyme will deactivate the enzyme.
Note 2: For enzyme activation, it is preferably activated at higher protein concentration. After activation, you may further dilute the enzyme.
3. Prepare working solutions:
3.1 Make MMP-3 Green substrate working solution: Dilute MMP-3 Green Substrate (Component A) with Assay Buffer (Component C) at 1:100 as shown in Table 1.
Table 1 MMP-3 Green substrate working solution for one 96-well plate (100 assays)
Components Volume
MMP-3 Green Substrate (Component A) 50 uL
Assay Buffer (Component C) 5 mL

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Total volume 5 mL
3.2 Make MMP-3 dilution: Dilute MMP-3 to an appropriate concentration in Assay Buffer (Component C) if purified MMP-3 is used.
Note: Pro-MMP-3 needs to be activated before use (see Step 2.2). Avoid vigorous vortexing of the enzyme.
3.3 Make inhibitors and compounds dilution: Make an appropriate concentration of known MMP-3 inhibitors and test compounds dilutions as desired if you are screening MMP-3 inhibitors.
4. Set up the enzymatic reaction in a 96-well microplate according to Table 2 and Table 3:
Table 2 Layout of the appropriate controls (as desired) and test samples in a 96-well microplate
SC SC
IC IC
VC VC
TC TC
TS TS
…. ….
…. ….
Note: SC= Substrate Control, IC= Inhibitor Control, VC=Vehicle Control, TC= Test Compound Control, TS=Test Samples.
Table 3 Reagent composition for each well
Substrate Control: Assay buffer, Total volume: 50 μL
Inhibitor Control: MMP-3 dilution and known MMP-3 inhibitor, Total volume: 50 μL
Vehicle Control:MMP-3 dilution and vehicle used to deliver test compound, Total volume: 50 μL
Test Compound Control*: MMP-3 containing assay buffer and test compound, Total volume: 50 μL
Test Sample: MMP-3 dilution with test compound, Total volume: 50 μL
Note 1: * Some strongly fluorescent test compounds may result in false-positive results.
Note 2: Make the total volume of all the controls to 50 μL for a 96-well plate or 20 μL for a 384-well plate by using Assay Buffer (Component C).
5. Run the enzyme reaction:
5.1 Pre-incubate the plate at a desired temperature for the enzyme reaction (e.g. 25 °C or 37 °C) for

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10-15 minutes if you are screening MMP-3 inhibitors.
5.2 Add 50 μL (96-well) or 20 μL (384-well) of MMP-3 Green substrate working solution (from Step 3.1) to the sample and control wells of the assay plate. Mix the reagents well.
5.3 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.

Analysis

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. In addition, a variety of data analysis can also be determined, e.g., determining inhibition %, EC50, IC50, etc.

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