

ab139451

MMP13 Inhibitor

Screening Assay Kit

(Fluorometric)

Instructions for Use

For the screening of MMP13 inhibitors

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1.	Background	3
2.	Principle of the Assay	3
3.	Protocol Summary	4
4.	Materials Supplied	5
5.	Storage and Stability	6
6.	Materials Required, Not Supplied	7
7.	Assay Protocol	8
8.	Data Analysis	12

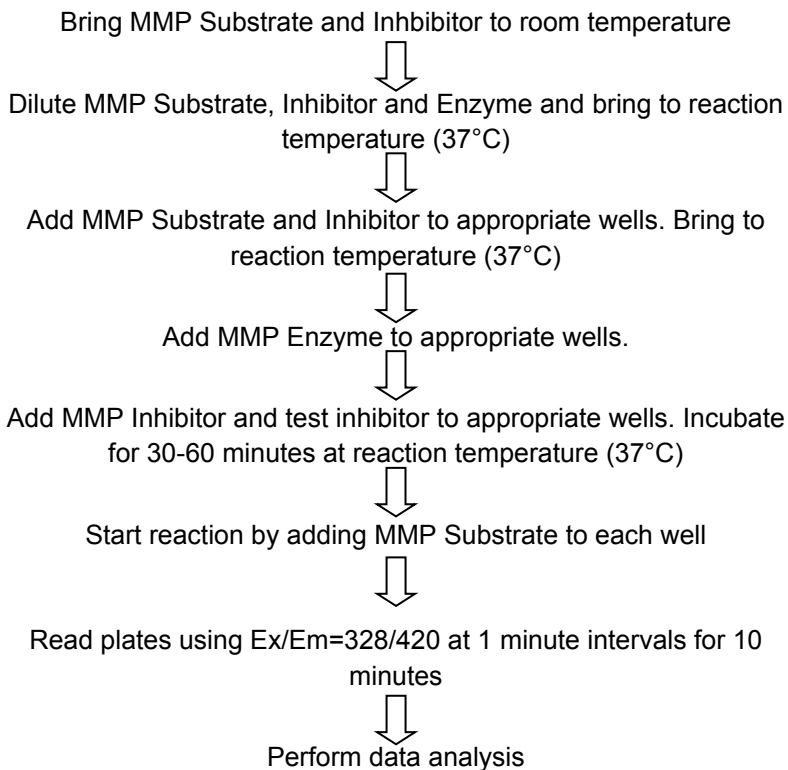
1. Background

Matrix metalloproteinase 13 (MMP13, collagenase-3) is a member of the MMP family of extracellular proteases. These enzymes play a role in many normal and disease states by virtue of their broad substrate specificities. Targets of MMP13 include collagen, gelatin, aggrecan, plasminogen, and CXCL12. MMP13 is secreted as a 60 kDa proenzyme (as measured by SDS-PAGE), and activated by cleavage to 48 kDa and below. MMP13 is an important target for inhibitor screening due to its involvement in diseases such as cancer and arthritis.

2. Principle of the Assay

Abcam MMP13 Inhibitor Screening Assay Kit (Fluorometric) (ab139451) is a complete assay system designed to screen MMP13 inhibitors using a quenched fluorogenic peptide: MMP Fluorogenic Substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH₂ [Mca=(7-methoxycoumarin-4-yl)-acetyl; Dpa=N-3-(2,4-dinitrophenyl)-L- α - β -diaminopropionyl]. Mca fluorescence is quenched by the Dpa group until cleavage by MMPs at the Gly-Leu bond separates the two moieties. The assays are performed in a convenient 96-well microplate format. The kit is useful to screen inhibitors of MMP13, a potential therapeutic target.

3. Protocol Summary



4. Materials Supplied

Item	Quantity	Storage
96-well Clear Microplate (½ Volume)	1	RT
MMP13 Enzyme (Human, Recombinant) 3.45 U/µL	1 x 53 µL	-80°C
MMP Fluorogenic Substrate 400 µM (437 µg/mL) in DMSO	1 x 200 µL	-80°C
MMP Inhibitor (1.3mM NNGH in DMSO)	1 x 50 µL	-80°C
MMP Calibration Standard 40 µM (17.8 µg/mL) in DMSO	1 x 50 µL	-20°C
Fluorometric Assay Buffer	1 x 20 mL	RT

5. Storage and Stability

- Store components as stated in table for the highest stability.
- The MMP13 enzyme should be handled carefully in order to retain maximal enzymatic activity. It is stable, in diluted or concentrated form, for several hours on ice.
- As supplied, MMP13 enzyme is stable for at least 5 freeze/thaw cycles. To minimize the number of freeze/thaw cycles, aliquot the MMP13 into separate tubes and store at -80°C.
- When setting up the assay, do not maintain diluted components at reaction temperature (e.g. 37°C) for an extended period of time prior to running the assay.
- One U MMP13 Enzyme = 100 pmol/min@ 37°C, 100 μ M thiopeptolide

6. Materials Required, Not Supplied

- Fluorescent microplate reader capable of excitation at 328 nm and emission at 420nm. The following Ex/Em have also been used: 320,340/393,400,405.
- Pipettes or multi-channel pipettes capable of pipetting 1-100 μ L accurately.
- Ice bucket to keep reagents cold until use.
- Water bath or incubator for component temperature equilibration.
- Orbital shaker

7. Assay Protocol

1. Briefly warm kit MMP substrate, MMP Calibration standard, and MMP Inhibitor to RT to thaw DMSO.
2. Dilute MMP Inhibitor 1/200 in assay buffer as follows. Add 1 μL inhibitor into 200 μL assay buffer, in a separate tube. Warm to reaction temperature (e.g. 37°C).
3. Thaw the DMSO stock vial of substrate and dilute sufficient volume to 40 μM in assay buffer (10 μL needed per well). Warm to reaction temperature (e.g. 37°C).
4. Dilute MMP13 enzyme 1/50 in assay buffer to required total volume (20 μL are needed per well). Warm to reaction temperature (e.g. 37°C) shortly before assay.
5. Pipette assay buffer into each desired well of the 1/2 volume microplate as follows:
 - Calibration = 80 μL in 3 wells (see step 11)
 - Control (no inhibitor) = 70 μL
 - MMP Inhibitor = 50 μL
 - Test inhibitor = varies (see Table 1)
6. Allow microplate to equilibrate to assay temperature (e.g. 37°C).

7. Add 20 μL MMP13 (diluted in step 4) to the control, MMP Inhibitor, and test inhibitor wells. Final amount of MMP13 will be 1.38 U per well (13.8 mU/ μL). Remember to not add MMP13 to the calibration wells!
8. Add 20 μL MMP Inhibitor (diluted in step 2) to the MMP Inhibitor wells only. Final NNGH concentration = 1.3 μM .
9. Add desired volume of test inhibitor to appropriate wells. See Table 1.
10. Incubate plate for 30-60 minutes at reaction temperature (e.g. 37°C) to allow inhibitor/enzyme interaction.
11. In the meantime, calibrate the fluorescent microplate reader, using Ex/Em=328/420: Prewarm assay buffer to reaction temperature in 3 wells in the microplate, then to each add 10 μL substrate peptide to give the concentration to be used in the assay (e.g., for 4 μM final add 10 μL 40 μM) and mix. When the fluorescent signal is constant, use this reading as the zero (Blank) value in arbitrary fluorescence units (RFUs). Using the same wells, with their mixtures of substrate peptide and buffer, add 10 μL calibration standard peptide to give 3 different final molar concentrations ranging between 2 and 10% of the substrate peptide molar concentration (e.g., 80, 200, and 400 nM) and measure their fluorescence. Use these values to build a standard curve relating micromolar concentration (x axis) to RFUs (y axis). The slope of the line is the conversion factor (CF). If multiple concentrations of substrate peptide are used, such as in kinetic

determinations, step 11 must be performed for each concentration, due to absorptive quenching by the substrate peptide. Note: this calibration can be done at any time.

12. Start reactions by the addition of 10 μL substrate (diluted and equilibrated to reaction temperature in step 3). Final substrate concentration = 4 μM .
13. Continuously read plates in the fluorescent microplate reader, using Ex/Em=328/420. For example, record data at 1 minute time intervals for 10 minutes.
14. Perform data analysis (see below).

NOTE: Retain microplate for future use of unused wells.

Table 1. Example of Samples

Sample	Assay Buffer	MMP13 (69 U//μL)	Inhibitor (6.5 μM)	Substrate (40 μM)	Total Volume
Control	70 μ L	20 μ L	0 μ L	10 μ L	100 μ L
MMP Inhibitor	50 μ L	20 μ L	20 μ L	10 μ L	100 μ L
Test Inhibitor*	X μ L	20 μ L	Y μ L	10 μ L	100 μ L

*Test inhibitor is the experimental inhibitor. Dissolve/dilute inhibitor into assay buffer and add to appropriate wells at desired volume “Y”. Adjust volume “X” to bring the total volume to 100 μ L.

Example of plate:

well#	sample
A1	Calibration
B1	Calibration
C1	Calibration
D1	Control
E1	Control
F1	MMP Inhibitor
G1	MMP Inhibitor
H1	Test inhibitor
A2...	Test inhibitor...

8. Data Analysis

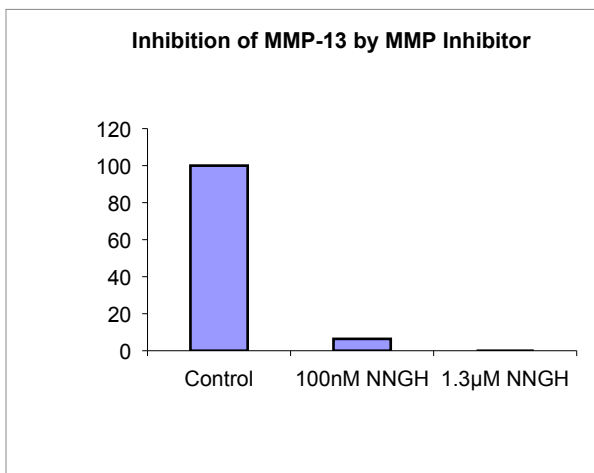
1. Plot data as RFUs (minus Blank RFU value determined during calibration, step 11) versus time for each sample.
2. Determine the range of initial time points during which the reaction is linear.
3. Obtain the initial reaction velocity (**V**) in RFUs/min: determine the slope of a line fit to the initial linear portion of the data plot using an appropriate routine.
4. It is best to use a range of inhibitor concentrations, each in duplicate. Average the slopes of duplicate samples.

A. To determine inhibitor % remaining activity:

$$\text{Inhibitor \% activity remaining} = (V_{\text{inhibitor}} / V_{\text{control}}) \times 100$$

See Figure 1 for example.

Figure 1. Inhibition of MMP13 by NNGH. Example of inhibitor data.



B. To determine the activity of the samples expressed as picomoles substrate hydrolyzed per minute:

$$X \text{ pmoles substrate/min} = 1/CF \times V \times \text{vol}$$

Where CF is the conversion factor (micromolar concentration/RFUs, from step 11), V is initial reaction velocity (RFUs/min, from step 17), and vol is the reaction volume in microliters (100).

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