

**ab139442**

**MMP12 Inhibitor**

**Screening Assay Kit**

**(Fluorometric)**

**Instructions for Use**

For the screening of MMP12 inhibitors

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



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# 1. Background

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Matrix metalloproteinase-12 (MMP12, metalloelastase, macrophage elastase, commonly confused with neutrophil elastase) 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 MMP12 include elastin, fibronectin, laminin, plasminogen, u-PAR, and tissue factor pathway inhibitor. MMP12 is secreted as a 53 kDa proenzyme (as measured by SDS-PAGE), and activated by cleavage to forms of 45-22 kDa. MMP12 is an important target for inhibitor screening due to its involvement in diseases such as cancer and emphysema.

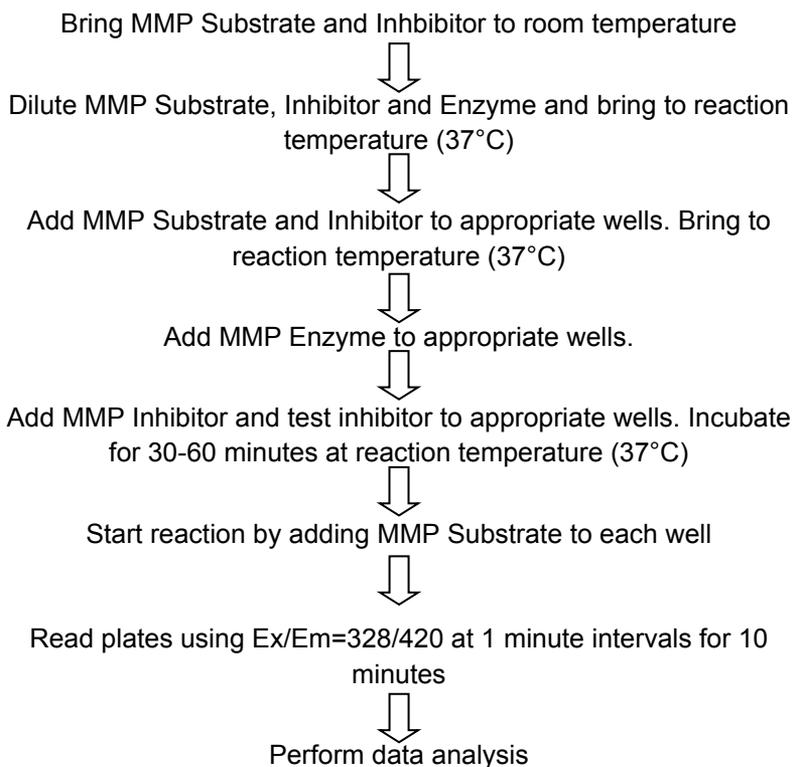
# 2. Principle of the Assay

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Abcam MMP12 Inhibitor Screening Assay Kit (Fluorometric) (ab139442) Kit is a complete assay system designed to screen MMP12 inhibitors using a quenched fluorogenic peptide: MMP Fluorogenic Substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH<sub>2</sub> [Mca = (7-methoxycoumarin-4-yl)-acetyl; Dpa=N-3-(2,4-dinitrophenyl)-L- $\alpha$ - $\beta$ -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 MMP12, a potential therapeutic target. The compound NNGH<sup>9</sup> is also included as a prototypic control inhibitor.

### 3. Protocol Summary

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## 4. Materials Supplied

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Item	Quantity	Storage
96-well Clear Microplate (½ Volume)	1	RT
MMP12 Enzyme (Human, Recombinant) (10 U/μL)	1 x 14 μL	-80°C
MMP Fluorogenic Substrate (437 μg/mL in DMSO)	1 x 200 μL	-80°C
MMP Inhibitor (1.3 mM in DMSO)	1 x 50 μL	-80°C
MMP Calibration Standard (17.8 μg/mL in DMSO)	1 x 50 μL	-80°C
Fluorometric Assay Buffer	1 x 20 mL	RT

## 5. Storage and Stability

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- Store all components except microplate and assay buffer (RT) at  $-80^{\circ}\text{C}$  for the highest stability.
- The MMP12 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, MMP12 enzyme is stable for 4 freeze/thaw cycles. To minimize the number of freeze/thaw cycles, aliquot the MMP12 into separate tubes and store at  $-80^{\circ}\text{C}$ .
- When setting up the assay, do not maintain diluted components at reaction temperature (e.g.  $37^{\circ}\text{C}$ ) for an extended period of time prior to running the assay.
- One unit is defined as the amount of enzyme that will hydrolyze  $100\ \mu\text{M}$  thiopeptolide Ac-PLG-[2-mercapto-4-methyl-pentanoyl]-LG-OC<sub>2</sub>H<sub>5</sub> at  $100\ \text{pmol}/\text{min}$  at  $37^{\circ}\text{C}$ .

## 6. Materials Required, Not Supplied

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- 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 10-100  $\mu\text{L}$  accurately.
- Ice bucket to keep reagents cold until use.
- Water bath or incubator for component temperature equilibration.

## 7. Assay Protocol

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1. Briefly warm kit components MMP Substrate, MMP Calibration Standard and MMP Inhibitor to RT to thaw DMSO.
2. Dilute MMP inhibitor (NNGH) 1/200 in Fluorometric Assay Buffer as follows. Add 1  $\mu\text{L}$  inhibitor into 200  $\mu\text{L}$  Fluorometric Assay Buffer, in a separate tube. Warm to reaction temperature (e.g. 37°C).
3. Thaw the DMSO stock vial of MMP Fluorogenic Substrate and dilute sufficient volume to 40  $\mu\text{M}$  in assay buffer (10  $\mu\text{L}$  needed per well). Warm to reaction temperature (e.g. 37°C).

4. Dilute MMP12 Enzyme (Human, Recombinant) 1/285 in Fluorometric Assay Buffer to required total volume (20  $\mu$ L 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  $\frac{1}{2}$  volume microplate as follows:

Calibration = 80  $\mu$ L in 3 wells (see step 11)

Control (no inhibitor) = 70  $\mu$ L

MMP Inhibitor = 50  $\mu$ L

Test inhibitor = varies (see Table 1)

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...

6. Allow microplate to equilibrate to assay temperature (e.g. 37°C).

7. Add 20  $\mu\text{L}$  MMP12 Enzyme (diluted in step 4) to the control, inhibitor and test inhibitor wells. Final amount of MMP12 will be 0.7 U per well (7.0 mU/ $\mu\text{L}$ ). Remember to not add MMP12 to the calibration wells!
8. Add 20  $\mu\text{L}$  MMP Inhibitor (diluted in step 2) to the inhibitor wells only. Final MMP Inhibitor concentration = 1.3  $\mu\text{M}$ .
9. Add desired volume of test inhibitor to appropriate wells. See Table 1, below.
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  $\mu\text{L}$  MMP Fluorogenic Substrate to give the concentration to be used in the assay (e.g., for 4  $\mu\text{M}$  final add 10  $\mu\text{L}$  40 $\mu\text{M}$ ) 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 MMP Fluorogenic Substrate and Fluorometric Assay Buffer, add 10 $\mu\text{l}$  MMP Calibration Standard to give 3 different final molar concentrations ranging between 2 and 10% of the MMP Fluorogenic Substrate molar concentration (e.g., 80, 200, and 400 nM) and measure their fluorescence. Use these values to build a standard curve relating micromolar MMP Calibration Standard 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 MMP Fluorogenic Substrate.

**NOTE:** This calibration can be done at any time.

12. Start reactions by the addition of 10  $\mu\text{L}$  MMP Fluorogenic Substrate (diluted and equilibrated to reaction temperature in step 3). Final substrate concentration = 4  $\mu\text{M}$ .
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**

<b>Sample</b>	<b>Assay Buffer</b>	<b>MMP12 (35 mU//<math>\mu</math>L)</b>	<b>Inhibitor (6.5 <math>\mu</math>M)</b>	<b>Substrate (40 <math>\mu</math>M)</b>	<b>Total Volume</b>
<b>Control</b>	70 $\mu$ L	20 $\mu$ L	0 $\mu$ L	10 $\mu$ L	100 $\mu$ L
<b>MMP Inhibitor</b>	50 $\mu$ L	20 $\mu$ L	20 $\mu$ L	10 $\mu$ L	100 $\mu$ L
<b>Test Inhibitor*</b>	X $\mu$ L	20 $\mu$ L	Y $\mu$ L	10 $\mu$ L	100 $\mu$ L

\*Test inhibitor is the experimental inhibitor. Dissolve/dilute inhibitor into Fluorometric Assay Buffer and add to appropriate wells at desired volume "Y". Adjust volume "X" to bring the total volume to 100  $\mu$ L.

## 8. Data Analysis

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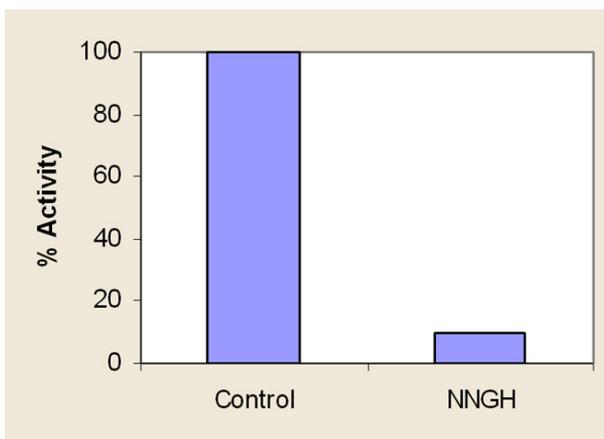
1. Plot data as RFUs (minus Blank RFU value determined during calibration, step 11) versus time for each sample.
2. Determine the range of time points during which the reaction is linear.
3. Obtain the initial reaction velocity ( $V$ ) in RFUs/min: determine the slope of a line fit to the initial linear 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 MMP12 by NNGH (100 nM). Example of Inhibitor Data.**



**B. To determine the activity of the samples expressed as picomols substrate hydrolyzed per minute**

Employ the following equation:

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

Where

CF is the conversion factor (micromolar concentration/RFUs (from Data Analysis, step 3).

$\mathbf{V}$  is initial reaction velocity (RFUs/min, from step 17).

vol. is the reaction volume in microliters (100  $\mu\text{L}$ ).

**UK, EU and ROW**

Email: [technical@abcam.com](mailto:technical@abcam.com) | Tel: +44-(0)1223-696000

**Austria**

Email: [wissenschaftlicherdienst@abcam.com](mailto:wissenschaftlicherdienst@abcam.com) | Tel: 019-288-259

**France**

Email: [supportscientifique@abcam.com](mailto:supportscientifique@abcam.com) | Tel: 01-46-94-62-96

**Germany**

Email: [wissenschaftlicherdienst@abcam.com](mailto:wissenschaftlicherdienst@abcam.com) | Tel: 030-896-779-154

**Spain**

Email: [soportecientifico@abcam.com](mailto:soportecientifico@abcam.com) | Tel: 911-146-554

**Switzerland**

Email: [technical@abcam.com](mailto:technical@abcam.com)

Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

**US and Latin America**

Email: [us.technical@abcam.com](mailto:us.technical@abcam.com) | Tel: 888-77-ABCAM (22226)

**Canada**

Email: [ca.technical@abcam.com](mailto:ca.technical@abcam.com) | Tel: 877-749-8807

**China and Asia Pacific**

Email: [hk.technical@abcam.com](mailto:hk.technical@abcam.com) | Tel: 108008523689 (中國聯通)

**Japan**

Email: [technical@abcam.co.jp](mailto:technical@abcam.co.jp) | Tel: +81-(0)3-6231-0940

[www.abcam.com](http://www.abcam.com) | [www.abcam.cn](http://www.abcam.cn) | [www.abcam.co.jp](http://www.abcam.co.jp)