ab308158 – MMP-12 Activity Assay Kit (Fluorometric)

A homogenous assay that allows the quantification of MMP-12 activity in tissues and sputum samples.
For research use only - not intended for diagnostic use.

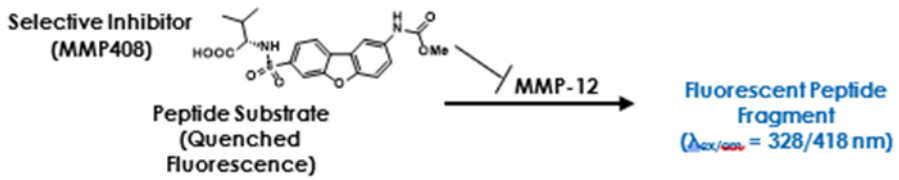
For overview, typical data and additional information please visit:

http://www.abcam.com/ab308158

Storage and Stability
The entire Assay kit may be stored at -20 °C and protected from light.

Introduction:

MMP-12, also known as macrophage elastase (EC 3.4.24.65) is a metalloprotease that hydrolyzes the fibrilar connective proteins of the extracellular matrix (ECM) such as elastin and collagen. Like all MMPs, MMP-12 is synthesized as an inactive pro-enzyme, which contains an autoinhibitory domain that interacts with the zinc coordination site in the catalytic domain and must be cleaved for enzymatic activity. MMP-12 is produced by the activated macrophages at sites of inflammation, particularly in pulmonary alveolar tissues. Cleavage of elastin and other ECM proteins play a pivotal role in the pathology of lung diseases, especially chronic obstructive pulmonary disease (COPD) and emphysema. MMP-12 is strongly expressed in chronic smokers with COPD. MMP-12 activity has also been implicated in the development of atherosclerosis and ischemic stroke due to catabolism of ECM surrounding fibrotic plaques. **The MMP-12 Activity Assay Kit** is a homogenous assay that allows the quantification of MMP-12 activity in tissues and sputum samples. The assay utilizes a synthetic peptide substrate bearing both a fluorophore and a fluorescence quencher. Upon cleavage by active MMP-12, the fluorophore-bearing peptide fragment is unquenched to produce a bright fluorescent signal measured at Ex/Em = 328/418 nm. A selective MMP-12 inhibitor is included in the kit for determination of MMP-12 activity in heterogeneous biological samples, where other MMP isozymes may contribute to peptide substrate cleavage. The inhibitor displays 100-fold selectivity for MMP-12 over other MMPs, ensuring targeted inhibition. A general protease inhibitor cocktail that inhibits non-metalloproteases in tissue homogenates is also included. The assay is simple, easy, rapid, and has a detection limit of 10 µU MMP-12 activity per well.



Materials Supplied

Item	Quantity
MMP-12 Assay Buffer	25 mL
MMP-12 Substrate	100 µl
MMP-12 Inhibitor (MMP408)	1 vial
Non-Metalloprotease Inhibitor Cocktail	1 vial
MMP-12 Positive	1 vial
MCA Standard	20 µl

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- White 96-well plates with flat bottom
- Multiwell microplate spectrofluorometer

Reagent Preparation

- Store the kit at -20 °C, protected from light.
- Briefly centrifuge all small vials prior to opening
- Read the entire protocol before performing the assay

MMP-12 Assay Buffer: Allow the MMP-12 Assay Buffer to warm to room temperature (RT) prior to use. Store at 4 °C

MMP-12 Substrate: Provided as a stock solution in DMSO. Divide into aliquots and store at -20 °C, protected from light. Prior to use, warm solution to RT and vortex thoroughly.

MMP-12 Inhibitor (MMP408): Reconstitute the vial with 110 µl ddH_2O to yield a 1 mM MMP-12 Inhibitor stock solution. Store at -20 °C protected from light. Stable for at least 3 freeze/thaw cycles.

Non-Metalloprotease Inhibitor Cocktail: Reconstitute the vial with 1000 µl ddH_2O. Divide into aliquots and store at -20 °C. Stable for at least 3 freeze/thaw cycles.

MMP-12 Positive Control: Reconstitute the vial with 110 µl MMP-12 Assay Buffer. Divide into aliquots and store at -20 °C. Avoid repeated freeze/thaw cycles.

MCA Standard: Provided as a 1 mM MCA Standard stock solution in DMSO. Warm solution to RT prior to use. Store at -20 °C, protected from light. Stable for at least 3 freeze/thaw cycles.

Sample Preparation

For **Mammalian Tissue Samples**: Prior to preparing samples, add Non-Metalloprotease Inhibitor Cocktail to MMP-12 Assay Buffer at a 1:100 ratio (for example, add 10 µl of Non-Metalloprotease Inhibitor Cocktail per ml of Assay Buffer) and incubate the buffer on ice. Homogenize fresh or frozen tissue with MMP-12 Assay Buffer with a Dounce homogenizer (Cat. #1998 or equivalent) on ice. We recommend using 500 µl MMP-12 Assay Buffer per 100 mg of wet tissue. Centrifuge

the homogenate at 4 °C for 10 min at 15,000 x *g*. Transfer the clarified supernatant to a fresh pre-chilled microfuge tube. Add 5-40 µl of clarified supernatant to desired well(s) in a white, flat-bottomed 96-well plate. Note: Tissue homogenates may be stored at -20 °C in aliquots for future experiments.

Additional Notes:

The sample volume and/or dilution factor required can vary based upon the nature of the sample. For unknown samples, we suggest doing a pilot experiment by testing several amounts to ensure the readings are within the range of the standard curve.

To quantify MMP-12 specific activity in terms of protein content tissue sample lysates, we recommend measuring sample protein concentration using protein quantitation Kit (BioVision Cat # K810) or any equivalent protein assay.

For Sputum and Bronchoalveolar lavage Samples: Collect sputum or bronchoalveolar fluid samples by any standard methods. Prior to preparing samples, add Non-Metalloprotease Inhibitor Cocktail to MMP-12 Assay Buffer at 1:100 ratio. For example, add 10 µl of Non-Metalloprotease Inhibitor Cocktail per ml of Assay Buffer and incubate on ice. Dilute sample at a 1:2 ratio with ice-cold MMP-12 Assay Buffer with Non-Metalloprotease Inhibitor and vortex thoroughly. Homogenize the diluted sample on ice using a Dounce homogenizer or probe sonicator until sample liquefaction occurs. Centrifuge the samples at 4 °C for 10 min at 15,000 x *g*. Transfer the clarified supernatant to a fresh pre-chilled microfuge tube. Add 5-40 µl of sample supernatant into the desired well(s).

For MMP-12 Positive Control: Thaw the reconstituted MMP-12 Positive Control aliquots and incubate solution at 37 °C for 15 min to allow MMP-12 autolytic activation (self-cleavage). Then add 10 µl of diluted MMP-12 Positive Control solution into the desired well(s).

Assay Reaction Set Up: Prepare assay reaction wells according to the table below. For each sample type, prepare at least two parallel sample wells labeled as **Test Sample** well and **Inhibitor Control (sample + 1 µM MMP408)** well. If desired, you may also prepare a **Background Control** well **(no enzyme)**.

Note: To prepare the MMP-12 Inhibitor (10 µM), dilute the 1 mM MMP-12 Inhibitor stock solution at 1:100 ratio in MMP-12 Assay Buffer.

	Test Sample	Inhibitor Control	Background Control	Positive Control
Test Sample	5-40 µl	5-40 µl	--	--
Positive Control	--	--	--	10 µl
MMP-12 Inhibitor (10 µM)	--	10 µl	--	--
MMP-12 Assay Buffer	to 50 µl	to 50 µl	to 50 µl	to 50 µl

MCA Standard Curve Preparation: Prepare a 50 µM MCA Standard working solution by adding 10 µl of the 1 mM Standard stock to 190 µl of MMP-12 Assay Buffer. Add 0, 2, 4, 6, 8, and 10 µl of the 50 µM Standard working solution into a series of wells to generate 0, 100, 200, 300, 400 and 500 pmol of Standard/well. Adjust the volume of all MCA Standard wells to 100 µl with MMP-12 Assay Buffer.

Assay Procedure

1. **Reaction Preparation:**
   Preincubate the plate for 15 min at 37 °C to allow for inhibitor binding and sample temperature to equilibrate. During the preincubation step, prepare MMP-12 Substrate working solution by diluting the MMP-12 Substrate stock solution with MMP-12 Assay Buffer at 1:100 ratio. Prepare 50 µl of substrate working solution for each reaction to be performed. For example, for 10 reaction wells, mix 5 µl of MMP-12 Substrate stock with 495 µl MMP-12 Assay Buffer.
   Start the reaction by adding 50 µl of the diluted substrate working solution to each reaction well, yielding a final volume of 100 µl per well. ***Do not add MMP-12 Substrate solution to the MCA Standard wells.***
2. **Measurement:** Measure the fluorescence at Ex/Em = 328/418 nm of all sample wells in kinetic mode for 60 min at 37 °C. We strongly recommend reading in kinetic mode in order to ensure that the measurements recorded are within the linear range of the reaction. Ideal measurement time for the linear range may vary depending upon the sample. Note: The MCA Standard curve wells should be read in endpoint mode (Ex/Em = 328/418 nm).
3. **Calculations**: Subtract the 0 pmole/well Standard reading from all of the Standard readings, plot the background-subtracted Standard values and calculate the slope of the MCA Standard Curve. For all reaction wells, choose two time points (t1 and t2) in the linear phase of the reaction progress curves and obtain the corresponding fluorescence values (RFU1 and RFU2). Determine the change in fluorescence over the time interval (ΔF = RFU2 – RFU1). Subtract the ΔF value of the Inhibitor Control (ΔFI) from that of the corresponding test sample (ΔFS) well to obtain the specific change in fluorescence intensity generated by MMP-12 activity (denoted by FC) for each sample:

$$F_C = \Delta F_S - \Delta F_I$$

Apply the F_C values to the MCA Standard Curve to get *B* pmole of substrate metabolized by MMP-12 during the reaction time:

$$\textbf{Sample MMP-12 Activity} = \frac{B}{\Delta T \times P} = \textbf{pmol/min/(ml or mg)}$$

Where: ***B*** is the amount of peptide substrate cleaved, calculated from the MCA Standard Curve (in pmole)
ΔT is the linear phase reaction time $t_2 - t_1$ (in min)
P is the amount of sample added to the well (in ml of biological fluid or mg of tissue/protein)

Note: Remember to account for any dilution of the sample made during sample preparation when determining ***P***.

MMP-12 Unit Definition: One unit of MMP-12 activity is the amount of enzyme that generates 1 µmole of unquenched 7-methoxycoumarin-4-acetate (MCA) peptide fragment per min by hydrolysis of 1 µmole peptide substrate at 37 °C and pH 7.5.

For technical support contact information, visit: www.abcam.com/contactus