ab112146

MMP Activity Assay Kit (Fluorometric - Green)

Instructions for Use

For detecting MMP activity in biological samples using our proprietary green fluorescence probe

View kit datasheet: www.abcam.com/ab112146
(use www.abcam.cn/ab112146 for China, or www.abcam.co.jp/ab112146 for Japan)

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

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1. Introduction

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.

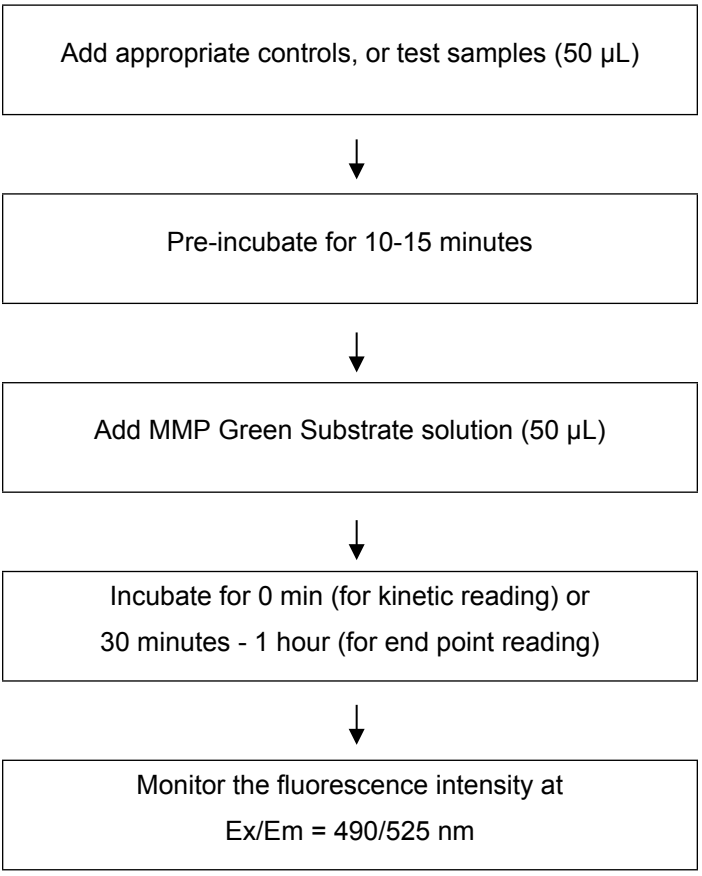
ab112146 MMP Activity Assay Kit uses a fluorescence resonance energy transfer (FRET) peptide as a generic MMP activity indicator. It is designed to check the general activity of an MMP enzyme and to screen MMP inhibitors. In the intact FRET peptide, the fluorescence of one part is quenched by another. After cleavage into two separate fragments by MMPs, the fluorescence is recovered. With excellent fluorescence quantum yield and longer wavelength, the probe is much more sensitive than an EDANS/Dabcyl FRET substrate. Its signal can be easily read by a fluorescence microplate reader at Ex/Em = 490/525 nm.

Kit Key Features

- **Convenient Format**: Includes all the key assay components
- **Optimized Performance:** Optimized conditions for the detection of generic MMP protease activity
- **Continuous:** Easily adapted to automation without a separation step.
- **Convenient:** Formulated to have minimal hands-on time. No wash step required.
- **Non-Radioactive:** No special requirements for waste treatment.

2. Protocol Summary

Summary for One 96-well Plate



Note: Thaw all the kit components to room temperature before starting the experiment.

3. Kit Contents

Components	Amount
Component A: MMP Green Substrate (light sensitive)	60 µL
Component B: APMA, 1M 4-Aminophenylmercuric Acetate	20 µL
Component C: Assay Buffer	20 mL

4. Storage and Handling

Store at -20 °C and keep from light.

Component C can be stored at 4 °C for convenience

5. Assay Protocol

Note: This protocol is for one 96 - well plate.

A. Preparation of Samples

Prepare MMP containing biological samples as desired.

B. Activation of pro-MMP:

1. Make 2mM APMA working solution (2x): Dilute 1 M APMA (Component B) with Assay Buffer (Component C) at 1:500 to get a 2 mM APMA working solution.

   Note: APMA belongs to organic mercury. Handle with care! Dispose it according to local regulations.

2. Incubate the MMPs with APMA working solution: Incubate the MMP containing-samples or purified MMPs with equal volume of 2 mM APMA working solution (2x, from Step B.1). For instance, use 25 µl of sample and add 25 µl of 2 mM APMA for a total volume of 50 µl per well. Refer to Appendix for incubation time. Activate MMPs immediately before the experiment.

   Note 1: 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.

C. Preparation of Working Solutions

1. Make MMP Green Substrate working solution: Dilute MMP Green Substrate (Component A) with Assay Buffer (Component C) at 1:100 as shown in Table 1.

Components	Volume
MMP Green Substrate (Component A)	50 µL
Assay Buffer (Component C)	5 mL
Total Volume	5.050 mL

Table 1. MMP Green Substrate working solution for one 96-well plate

2. Make MMP dilution: Dilute MMPs to an appropriate concentration in Assay Buffer (Component C) if purified MMP is used.

   Note: Pro-MMP needs to be activated before use (see Step B.2). Avoid vigorous vortexing of the enzyme

3. Make inhibitors and compounds dilution: Make dilutions of known MMPs inhibitors and test compounds as desired if you are screening MMPs inhibitors.

D. Set up the enzymatic reaction in a 96-well microplate according to Table 2 and Table 3:

SC	SC	.....	.....
IC	IC	....	....
VC	VC	....	....
TC	TC		
TS	TS		
.....	.....		
.....	.....		
.....	.....		

Table 2. Layout of appropriate controls (as desired) and test samples in a 96-well microplate. *Note: SC= Substrate Control, IC= Inhibitor Control, VC=Vehicle Control, TC= Test Compound Control, TS=Test Samples.*

Identifier	Contents	Total Volume
Substrate Control	Assay Buffer	50 µL
Inhibitor Control	MMP Dilution + known MMPs Inhibitor	50 µL
Vehicle Control	MMP dilution and vehicle used to deliver test compound	50 µL
Test Compound Control*	MMP containing assay buffer and test compound	50 µL
Test Sample	MMP dilution with test compound	50 µL

Table 3. Reagent composition for each well.

*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).

E. Run Enzyme Reaction:

1. 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.

2. Add 50 µL (96-well) or 20 µL (384-well) of MMP Green Substrate working solution (from Step C.1) to the sample and control wells of the assay plate. Mix the reagents well.

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.

6. Data 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 (as shown in Figure 1). In addition, a variety of data analysis can also be determined, e.g., determining inhibition %, EC_{50}, IC_{50}, etc

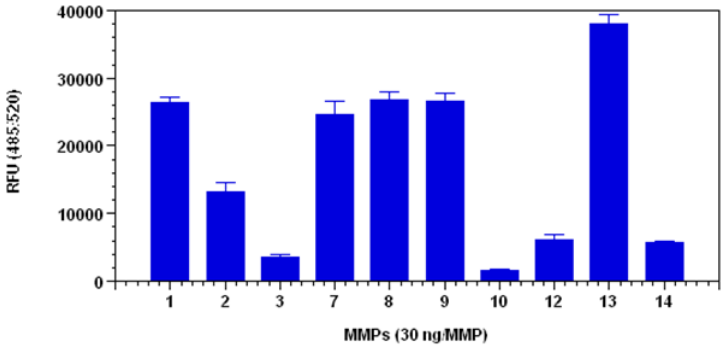


Figure 1. Detecting the activity of MMPs using ab112146. The APMA-activated MMPs, 30 ng each, were mixed with MMP Green Substrate. The fluorescence signal was monitored one hour after starting the reaction by using a microplate reader with a filter set of Ex/Em = 490/525 nm. The reading from all wells was subtracted with the reading from substrate control, which contains MMP Green Substrate but no MMPs. Although different MMPs showed different cleavage rate on this substrate, the MMP Green Substrate can detect the activity of sub-nanogram of all MMPs (n=3).

7. Appendix

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 hr.
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.

8. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)*** or ***Deproteinizing sample preparation kit (ab93299)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

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