

ab112147

**MMP Activity Assay Kit
(Fluorometric - Red)**

Instructions for Use

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

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

Table of Contents

1. Introduction	3
2. Protocol Summary	5
3. Kit Contents	6
4. Storage and Handling	6
5. Assay Protocol	7
6. Data Analysis	12
7. Appendix	13
8. Troubleshooting	14

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.

ab112147 uses a fluorescence resonance energy transfer (FRET) peptide as a MMP substrate. In the intact FRET peptide, the fluorescence of one part is quenched by the other. Upon cleavage into two separate fragments by MMPs, the fluorescence is recovered. ab112147 is designed to check the general activity of a MMP enzyme. It can also be used to screen MMP inhibitors when a purified MMP enzyme is used.

With excellent fluorescence quantum yield and longer wavelength, this substrate shows less interference from autofluorescence of test compounds and cellular components and is much more sensitive than an EDANS/Dabcyl FRET substrate. Its signal can be easily read by a fluorescence microplate reader at Ex/Em = 540/590 nm. The pH-independent fluorescence makes the assay reading available for the whole physiological pH range. The high

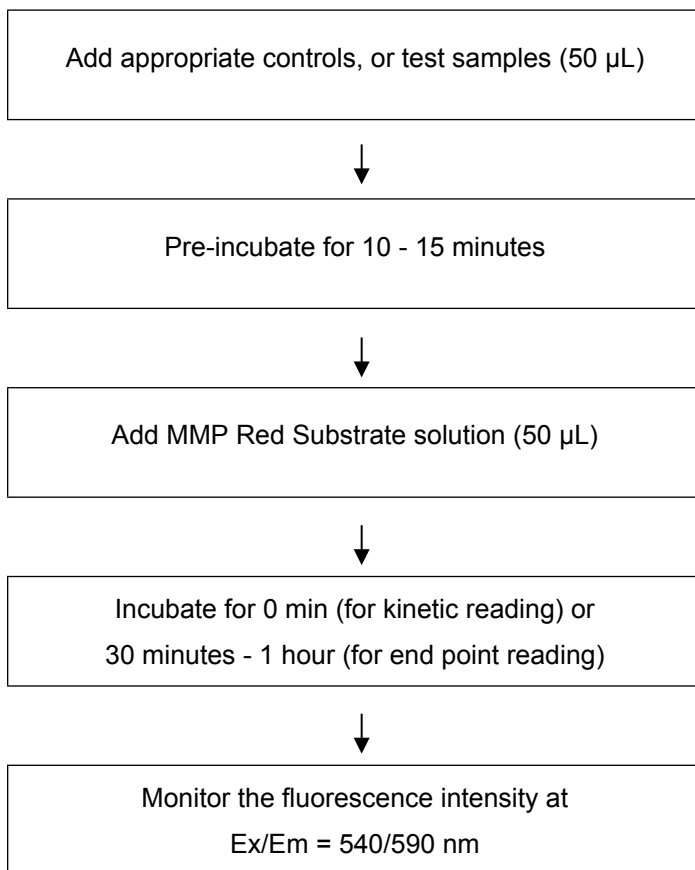
photostability of this FRET peptide provides a useful imaging probe. Many labs have used this kit for the high throughput screening of MMP inhibitors as potential anticancer drug candidates. This assay might be also used for monitoring cancer cells.

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 is 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 Red Substrate (light sensitive)	60 μ L
Component B: APMA, 1M 4-Aminophenylmercuric Acetate	20 μ L
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-MMPs:

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 MMPs containing-samples or purified MMPs with equal volume of 2 mM APMA working solution (2x, from Step B.1). For instance, use 25 μ l of sample and add 25 μ l of 2 mM APMA for a total volume of 50 μ l 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 Red Substrate working solution: Dilute MMP Red Substrate (Component A) with Assay Buffer (Component C) at 1:100 as shown in Table 1.

Components	Volume
MMP Red Substrate (Component A)	50 μ L
Assay Buffer (Component C)	5 mL
Total Volume	5.050 mL

Table 1. MMP Red Substrate working solution for one 96-well plate (100 assays)

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 dilutions 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 μ L
Inhibitor Control	MMP Dilution + known MMPs Inhibitor	50 μ L
Vehicle Control	MMP dilution and vehicle used to deliver test compound	50 μ L
Test Compound Control*	MMP containing assay buffer and test compound	50 μ L
Test Sample	MMP dilution with test compound	50 μ L

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 μ L for a 96-well plate or 20 μ L 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 Red 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 = 540/590 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

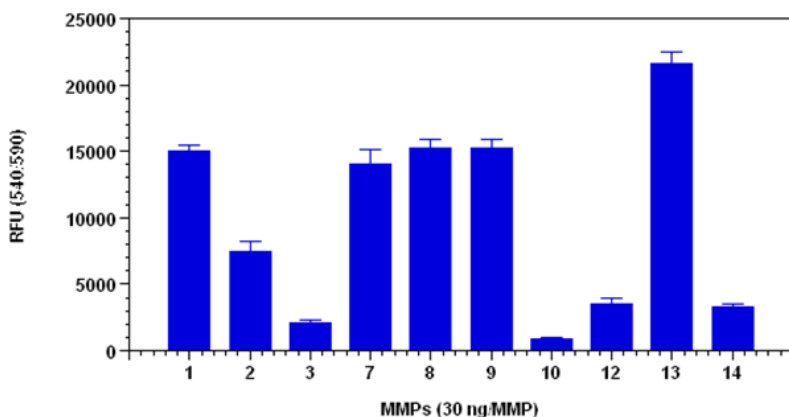


Figure 1. Detect the activity of MMPs using ab112147. The APMA-activated MMPs, 30 ng each, were mixed with MMP Red Substrate. The fluorescence signal was monitored one hour after the start of the reaction by using a microplate reader with a filter set of Ex/Em = 540/590 nm. The reading from all wells was subtracted with the reading from substrate control, which contains MMP Red Substrate but no MMPs. Although different MMPs showed different cleavage rate on this substrate, the MMP Red 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)

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).

UK, EU and ROW

Email:

technical@abcam.com

Tel: +44 (0)1223 696000

www.abcam.com

US, Canada and Latin America

Email: us.technical@abcam.com

Tel: 888-77-ABCAM (22226)

www.abcam.com

China and Asia Pacific

Email: hk.technical@abcam.com

Tel: 400 921 0189 / +86 21 2070 0500

www.abcam.cn

Japan

Email: technical@abcam.co.jp

Tel: +81-(0)3-6231-0940

www.abcam.co.jp