ab118972

MMP-3 Activity Assay Kit (Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of MMP-3 activity in various samples.

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 3a, Last updated 15 August 2023
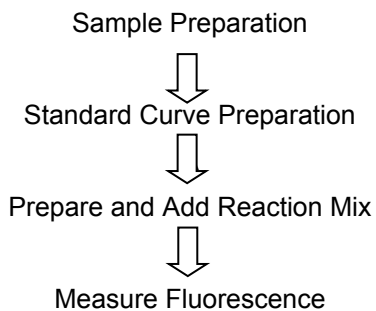
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1.   Overview

The matrix metalloproteinase-3 (MMP-3, stromelysin-1) exhibits a number of activities that would make it a particularly good tumor promoter. Like several other MMPs, MMP-3 was first cloned and later recloned as a cancer-specific gene. In addition to degrading numerous extracellular matrix components, MMP-3 can activate gelatinase B, the collagenases and several serpin-type serine proteinase inhibitors. Moreover, it can release a number of cell surface molecules, including E-cadherin, a known contributor to cancer development.

In Abcam's MMP-3 Activity Assay Kit, MMP-3 hydrolyzes a specific FRET substrate to release the quenched fluorescent group Mca, which can be detected fluorometrically at Ex/Em=325/393 nm. The kit provides a rapid, simple, sensitive and reliable test which can also be used as a high throughput assay of MMP-3 activity.

The assay sensitivity is < 50 µU.

2.   Protocol Summary

Sample Preparation

⇩

Standard Curve Preparation

⇩

Prepare and Add Reaction Mix

⇩

Measure Fluorescence

3.   Materials Supplied

Item	Quantity
MMP-1/3 Assay Buffer/MMP-3 Assay Buffer	25 mL
MMP-3 Substrate	200 µL
MCA Peptide Standard/Mca Standard (1 mM)	20 µL
MMP-3 Positive Control/MMP-3 Positive Control (Lyophilized)	1 vial

4.   Storage and Stability

Upon arrival, store kit at -20°C, protected from light. Read the entire protocol before performing the assay.

Allow Assay Buffer to warm to room temperature.

Briefly centrifuge vials prior to opening.

MMP-3 POSITIVE CONTROL: Reconstitute with 100 µl assay buffer. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Use within one week.

5.   Materials Required, Not Supplied

- Microcentrifuge

- Pipettes and pipette tips

- Fluorescent microplate reader

- 96-well plate

- Orbital shaker

6.   Assay Protocol

1. Sample Preparation:

a. For liquid samples (cell culture media, cell culture supernatant, milk, plasm, serum, urine and other biological fluids): Samples can be directly tested or after dilution in Assay Buffer. You might want to test different sample volumes to find the optimal that will give you a reading within the linear range of the standard curve.

b. For tissue or cell samples: Tissues (50 mg) or cells (1×10^6) can be homogenized in ~200 µl ice-cold MMP-1/3 Assay

Buffer/MMP-3 Assay Buffer then centrifuged to remove insoluble material at 13,000 x g for 10 minutes.

Prepare test samples to a total volume of 50 µl/well with Assay Buffer in a 96-well plate.

c. **Positive control:** dilute 5 – 10 µl of reconstituted MMP-3 Positive Control in Assay buffer to a total volume of 50 µl/well.

We suggest testing several doses of your sample to make sure readings are within the standard curve.

2. Standard Curve Preparation:
Dilute 5 µl of MCA Peptide Standard/1mM MMP Mca Standard in 495 µl MMP-1/3 Assay Buffer/MMP-3 Assay Buffer to generate a 10 µM standard solution.
Add 0, 10, 20, 30, 40, 50 µl to each well individually.
Adjust to a final volume of 100 µl/well with Assay Buffer to generate 0, 0.1, 0.2, 0.3, 0.4, 0.5 nmol/well of MCA Peptide Standard/Mca Standard.

3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix:

MMP-1/3 Assay Buffer/MMP-3 Assay Buffer	48 µl
MMP-3 substrate	2 µl

Add 50 µl of the Reaction Mix to each well containing the samples and positive controls. Mix well.

4. Measurement: Read Ex/Em = 325/393 nm R_1 at T_1. Read R_2 again at T_2 after incubating the reaction at room temperature for 60 min (or incubate longer time if the sample activity is low), protect from light.

The RFU of fluorescence generated by hydrolyzation of the substrate is:

$$\Delta RFU = R_2 - R_1$$

Note:

It is recommended to read kinetically to choose the R_1 and R_2 values that fall within the linear range of the Standard Curve.

7. Data Analysis

Subtract the zero Standard from the Standard readings.

Plot the Standard Curve and apply the ΔRFU to the standard curve to get B nmol of unquenched Mca generated between T_1 and T_2.

$$\text{MMP-3 Activity} = \frac{(B \times \text{Dilution Factor})}{(T_2 - T_1) \times V} = \text{nmol/min/ml} = \text{mU/ml}$$

Where:

**B** is the Mca amount (nmol) from MMP Mca Standard Curve

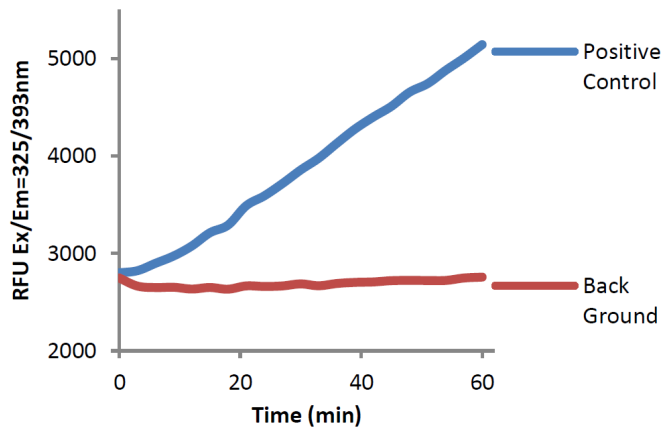**T1** is the time (min) of the first reading (R_1)

T2 is the time (min) of the second reading (R_2)

V is the pretreated sample volume (ml) added into the reaction well

Unit Definition: One unit is defined as the amount of enzyme that will generate 1.0 µmol of unquenched Mca per minute at room temperature.

MMP-3 Mca Standard

$y = 8.964x - 73$
$R^2 = 0.9974$
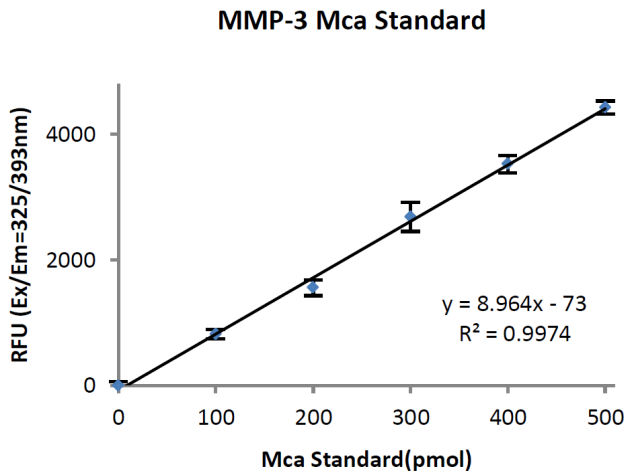
MMP-3 Positive Control

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

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)*
	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).

Technical Support

For all technical or commercial enquiries please go to: www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)