

MMP Activity Assay Kit (Fluorometric - Green) ab112146

★★★★★ [6 Abreviews](#) [41 References](#) [8 Images](#)

Overview

Product name	MMP Activity Assay Kit (Fluorometric - Green)
Detection method	Fluorescent
Sample type	Cell culture supernatant, Purified protein
Assay time	0h 60m
Product overview	MMP Activity Assay Kit ab112146 is designed to check the general activity of an MMP enzyme and to screen MMP inhibitors.

The MMP assay protocol uses a fluorescence resonance energy transfer (FRET) peptide as a generic MMP activity indicator. 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. The probe signal can be easily read by a fluorescence microplate reader at Ex/Em = 490/525 nm.

MMP activity assay protocol summary:

- activate pro-MMPs with APMA for between 20 min and 24 hrs (depending on which MMP is of interest)
- add samples to wells
- add MMP Green substrate
- analyze with a fluorescent microplate reader, either after 30-60 mins for end-point reading, or every 5 min for 30-60 min for kinetic reading

Notes **The kit measures total MMP activity. It does not give an individual read-out for each MMP. The substrate peptide is a sequence that is recognized by all MMPs. A specific inhibitor would need to be included to determine the activity of a specific MMP enzyme.**

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It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

Platform Microplate reader

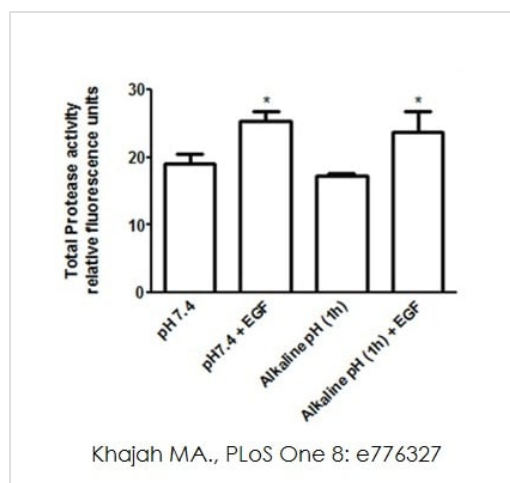
Properties

Storage instructions

Store at -20°C. Please refer to protocols.

Components	100 tests
APMA, 4-Aminophenylmercuric Acetate	1 x 20µl
Assay Buffer	1 x 20ml
MMP Green Substrate	1 x 60µl

Images

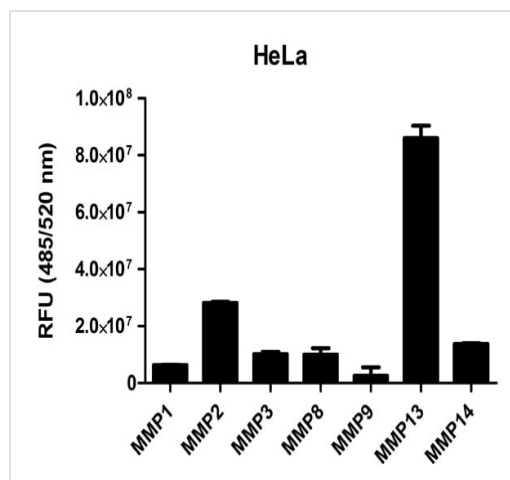


Khajah et al investigates endocrine resistant breast cancer cells in response to changes in extracellular pH. MMP activity of pII cells was determined using ab112146. Cells were cultured in a gassed or ungassed incubator for 1 hour and treated with EGF at 50ng/ml for 30 minutes. MMP activity was then determined using fluorogenic substrate. Fluorescence was measured for excitation/emission of 490/525 nm.

Functional studies - MMP Activity assay kit

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Khajah MA et al., PLoS One 8(10), Fig 8e. doi: 10.1371/journal.pone.0076327. Reproduced under the Creative Commons license <http://creativecommons.org/licenses/by/4.0/>



The tests show the MMP activities in different matrices. 25 µL (0.31 µg) APMA-activated MMPs were spiked into 25 µl (6.25e4 cells) of cell lysate. Cells (1e6-5e7 cells) were sonicated in 0.3 mL RIPA buffer with protease inhibitors. Protein amount was determined by BCA method.

Fluorescence shown after subtraction of vehicle control (duplicates, +/- SD).

Purified MMPs used:

MMP1 – [ab134442](#)

MMP2 – [ab125181](#)

MMP3 – [ab96555](#)

MMP8 – [ab168050](#)

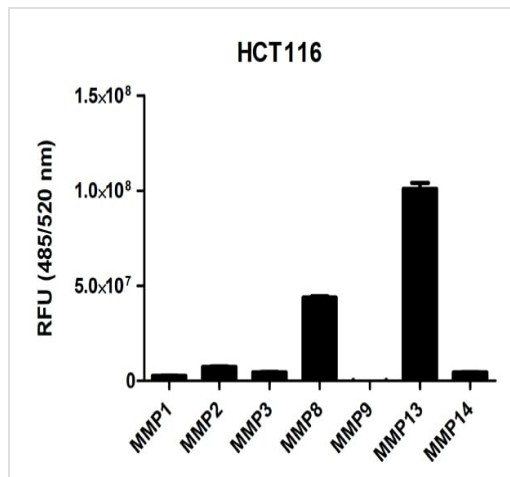
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MMP9 – [ab157344](#)

MMP13 – [ab134452](#)

MMP14 – [ab157068](#)



Functional Studies - MMP Activity Assay Kit
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The tests show the MMP activities in different matrices. 25 μ L (0.31 μ g) APMA-activated MMPs were spiked into 25 μ L (6.25e4 cells) of cell lysate. Cells (1e6-5e7 cells) were sonicated in 0.3 mL RIPA buffer with protease inhibitors. Protein amount was determined by BCA method. Fluorescence shown after subtraction of vehicle control (duplicates, +/- SD).

Purified MMPs used:

MMP1 – [ab134442](#)

MMP2 – [ab125181](#)

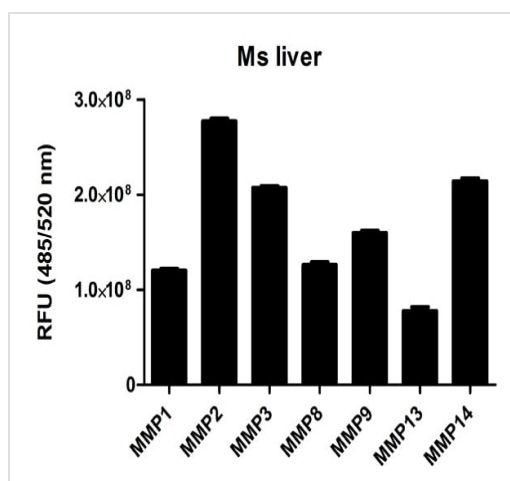
MMP3 – [ab96555](#)

MMP8 – [ab168050](#)

MMP9 – [ab157344](#)

MMP13 – [ab134452](#)

MMP14 – [ab157068](#)



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The tests show the MMP activities in different matrices. 25 μ L (0.31 μ g) APMA-activated MMPs were spiked into 25 μ L (0.56 mg) of mouse liver tissue lysate. Frozen tissue was homogenised in 1.2 mL RIPA buffer with protease inhibitors with 1 mm glass beads in a bead beater. Protein was determined by BCA method.

Fluorescence shown after subtraction of vehicle control (duplicates, +/- SD).

Purified MMPs used:

MMP1 – [ab134442](#)

MMP2 – [ab125181](#)

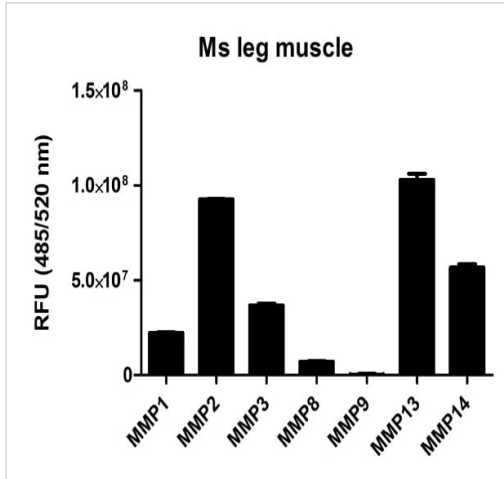
MMP3 – [ab96555](#)

MMP8 – [ab168050](#)

MMP9 – [ab157344](#)

MMP13 – [ab134452](#)

MMP14 – [ab157068](#)



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The tests show the MMP activities in different matrices. 25 uL (0.31 ug) APMA-activated MMPs were spiked into 25 ul (0.21 mg) of mouse leg muscle tissue lysate. Frozen tissue was homogenised in 1.2 ml RIPA buffer with protease inhibitors with 1 mm glass beads in a bead beater. Protein was determined by BCA method. Fluorescence shown after subtraction of vehicle control (duplicates, +/- SD).

Purified MMPs used:

MMP1 – [ab134442](#)

MMP2 – [ab125181](#)

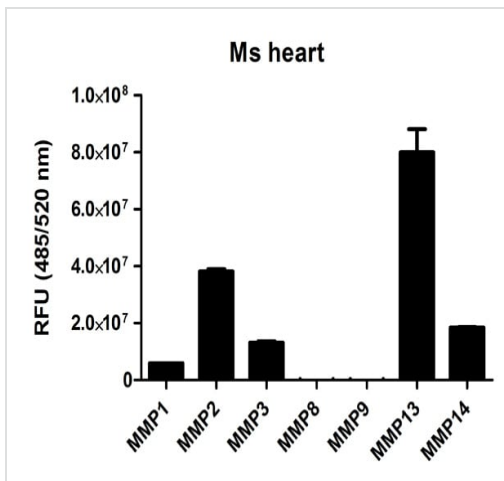
MMP3 – [ab96555](#)

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MMP9 – [ab157344](#)

MMP13 – [ab134452](#)

MMP14 – [ab157068](#)



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The tests show the MMP activities in different matrices. 25 uL (0.31 ug) APMA-activated MMPs were spiked into 25 ul (0.17 mg) of mouse heart tissue lysate. Frozen tissue was homogenised in 1.2 ml RIPA buffer with protease inhibitors with 1 mm glass beads in a bead beater. Protein was determined by BCA method. Fluorescence shown after subtraction of vehicle control (duplicates, +/- SD).

Purified MMPs used:

MMP1 – [ab134442](#)

MMP2 – [ab125181](#)

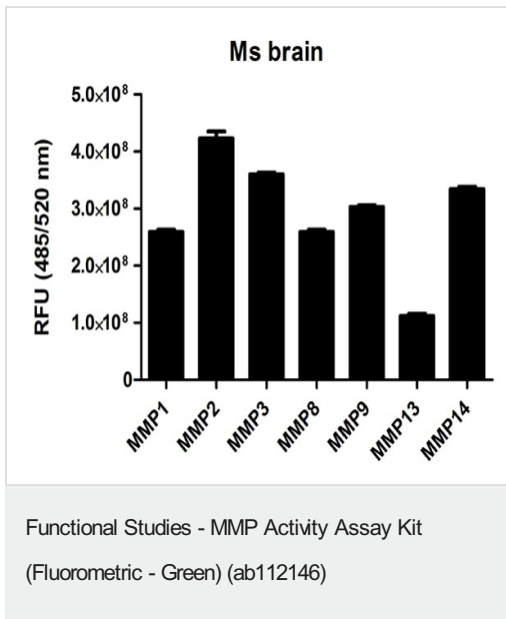
MMP3 – [ab96555](#)

MMP8 – [ab168050](#)

MMP9 – [ab157344](#)

MMP13 – [ab134452](#)

MMP14 – [ab157068](#)



The tests show the MMP activities in different matrices. 25 μ L (0.31 μ g) APMA-activated MMPs were spiked into 25 μ L (0.80 mg) of mouse brain tissue lysate. Frozen tissue was homogenised in 1.2 ml RIPA buffer with protease inhibitors with 1 mm glass beads in a bead beater. Protein was determined by BCA method. Fluorescence shown after subtraction of vehicle control (duplicates, +/- SD).

Purified MMPs used:

MMP1 – [ab134442](#)

MMP2 – [ab125181](#)

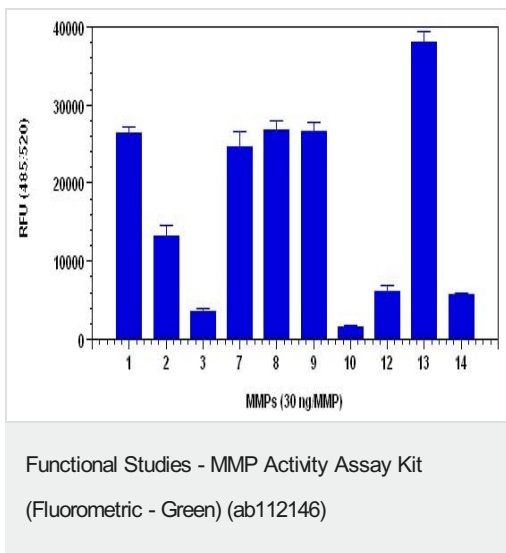
MMP3 – [ab96555](#)

MMP8 – [ab168050](#)

MMP9 – [ab157344](#)

MMP13 – [ab134452](#)

MMP14 – [ab157068](#)



Detection of activity of MMPs using ab112146.

APMA-activated purified MMPs (30 ng each) were mixed with Green substrate. The fluorescence signal was monitored after 1 hour by using a microplate reader at Ex/Em = 490/525 nm. The reading from all wells was subtracted with the reading from substrate control. Although different MMPs showed different cleavage rate on this MMP substrate, the MMP Green substrate can detect the activity of sub-nanogram of all MMPs (n=3).

NOTE: distinct purified MMP enzymes were used in this test. When using cell extracts, the kit will only detect a general MMP activity and it will not differentiate between the different MMPs.

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