abcam

Product datasheet

MMP Activity Assay Kit (Fluorometric - Green) ab112146

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概述

产品名称 MMP Activity Assay试剂盒(Fluorometric - Green)

检测方法 Fluorescent

样品类型 Cell culture supernatant, Purified protein

检测时间 0h 60m

产品概述 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

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 inihibitor would need to be included to determine the activity of a specific MMP enzyme.

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances.

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.

平台 Microplate reader

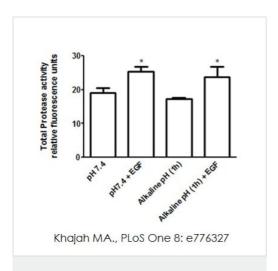
说明

存放说明

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

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

图片

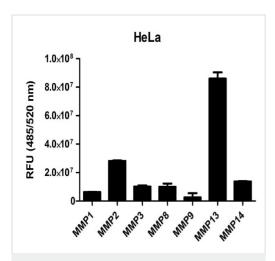


Khajah et al investigates endocrine resistant breast cancer cells in response to changes in extracellular pH. MMP activity of pll cells was determined using ab112146. Cells were cultured in a gassed or ungasssed incubator for 1 hour and treated with EGF at 50ng/ml for 30 minutes. MMP activity was then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

Functional studies - MMP Activity assay kit

(Flurometric - Green) (ab112146)

Khajah MAet 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/



Functional Studies - MMP Activity Assay Kit (Fluorometric - Green) (ab112146)

The tests show the MMP activities in different matrices. 25 uL (0.31 ug) APMA-activated MMPs were spiked into 25 ul (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 – <u>ab125181</u>

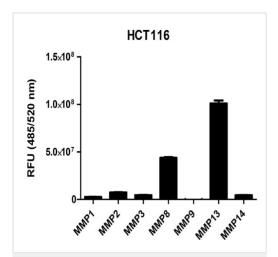
MMP3 - ab96555

MMP8 - ab168050

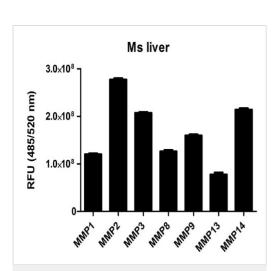
MMP9 - ab157344

MMP13 - ab134452

MMP14 - <u>ab157068</u>



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MMP2 - ab125181

MMP3 - ab96555

MMP8 - ab168050

MMP9 – **ab157344**

MMP13 - ab134452

MMP14 - ab157068

The tests show the MMP activities in different matrices. 25 uL (0.31 ug) APMA-activated MMPs were spiked into 25 ul (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

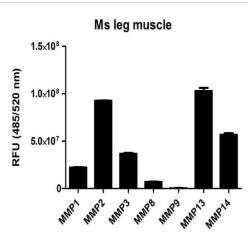
MMP3 - ab96555

MMP8 - ab168050

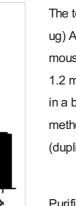
MMP9 - <u>ab157344</u>

MMP13 - ab134452

MMP14 - ab157068



Functional Studies - MMP Activity Assay Kit (Fluorometric - Green) (ab112146)



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

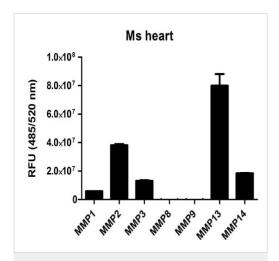
MMP3 - ab96555

MMP8 - ab168050

MMP9 - ab157344

MMP13 - ab134452

MMP14 - ab157068



Functional Studies - MMP Activity Assay Kit (Fluorometric - Green) (ab112146)

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

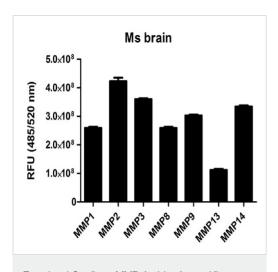
MMP3 - ab96555

MMP8 - ab168050

MMP9 - <u>ab157344</u>

MMP13 - ab134452

MMP14 - ab157068



Functional Studies - MMP Activity Assay Kit (Fluorometric - Green) (ab112146) The tests show the MMP activities in different matrices. 25 uL (0.31 ug) APMA-activated MMPs were spiked into 25 ul (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

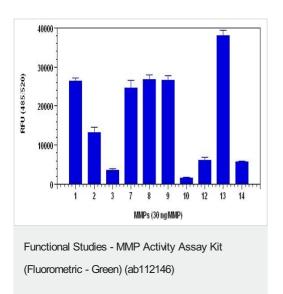
MMP3 - ab96555

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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