abcam

Product datasheet

MMP3 Inhibitor Screening Assay Kit (Colorimetric) ab139439

4 Images

Overview

Product name	MMP3 Inhibitor Screening Assay Kit (Colorimetric)
Detection method	Colorimetric
Sample type	Inhibitor compounds
Assay type	Enzyme activity
Product overview	Abcam's MMP3 Inhibitor Screening Assay Kit (Colorimetric) (ab139439) is a complete assay system designed to screen MMP3 inhibitors using a thiopeptide as a chromogenic substrate (Ac-PLG-[2-mercapto-4-methyl-pentanoyl]-LG-OC_2H5). The MMP cleavage site peptide bond is replaced by a thioester bond in the thiopeptide. Hydrolysis of this bond by an MMP produces a sulfhydryl group, which reacts with DTNB [5,5'-dithiobis(2-nitrobenzoic acid), Ellman's reagent] to form 2-nitro-5-thiobenzoic acid, which can be detected by its absorbance at 412 nm (ε=13,600 $M^{-1}cm^{-1}$ at pH 6.0 and above). The assays are performed in a convenient 96-well microplate format
Notes	This kit is useful to screen inhibitors of MMP3, a potential therapeutic target. The MMP inhibitor NNGH is also included as a prototypic control inhibitor. Thiol inhibitors should not be used with this kit, as they may interfere with the colorimetric assay.
Platform	Microplate reader

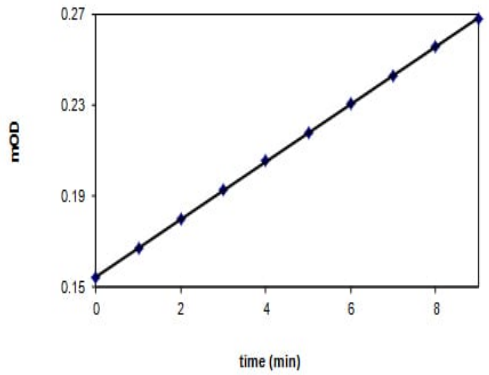
Properties

Storage instructions	Please refer to protocols.

Components	1 x 96 tests
96-well Clear Microplate (1/2 Volume)	1 unit
Colorimetric Assay Buffer	1 x 20ml
MMP Inhibitor	1 x 50µl
MMP Substrate	1 x 50µl
MMP3 Enzyme (Human, Recombinant)	1 x 30µl

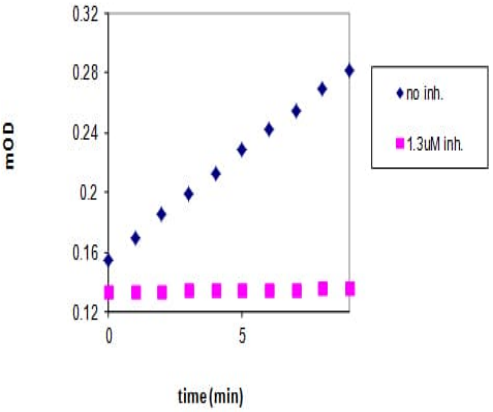
Function	Can degrade fibronectin, laminin, gelatins of type I, III, IV, and V; collagens III, IV, X, and IX, and cartilage proteoglycans. Activates procollagenase.
Sequence similarities	Belongs to the peptidase M10A family. Contains 4 hemopexin-like domains.
Domain	The conserved cysteine present in the cysteine-switch motif binds the catalytic zinc ion, thus inhibiting the enzyme. The dissociation of the cysteine from the zinc ion upon the activation-peptide release activates the enzyme.
Cellular localization	Secreted > extracellular space > extracellular matrix.

Images



Plot of OD vs. time

Slope = V= 1.185E-02 OD/min

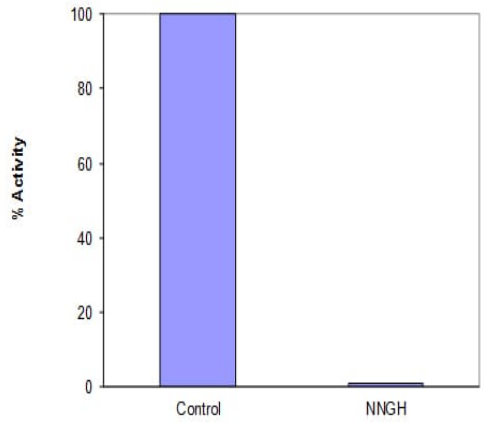


Inhibition of MMP3 by NNGH (1.3µM)

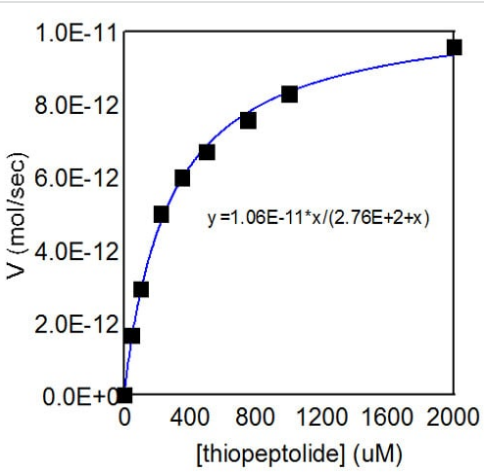
control slope = 1.4E-02 OD/min

inhibitor slope = 1.3E-04 OD/min

inhibitor % activity remaining = (1.3E-04/1.4E-02) x 100 = 0.9%



Inhibiton of MMP3 by NNGH



Example graph for Km and Vmax determination

Km = 276 µM

Vmax = 10.6 pmol/sec

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