abcam

Product datasheet

MMP14 Inhibitor Screening Assay Kit (Colorimetric) ab139454

4 Images

Overview

Product name	MMP14 Inhibitor Screening Assay Kit (Colorimetric)
Detection method	Colorimetric
Sample type	Inhibitor compounds
Assay type	Enzyme activity
Product overview	Abcam MMP14 Inhibitor Screening Assay Kit (Colorimetric) (ab139454) is a complete assay system designed to screen MMP14 inhibitors using a thiopeptide as a chromogenic substrate (Ac-PLG-[2-mercapto-4-methyl-pentanoyl]-LG-OC_2H_5). 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 MMP14, 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

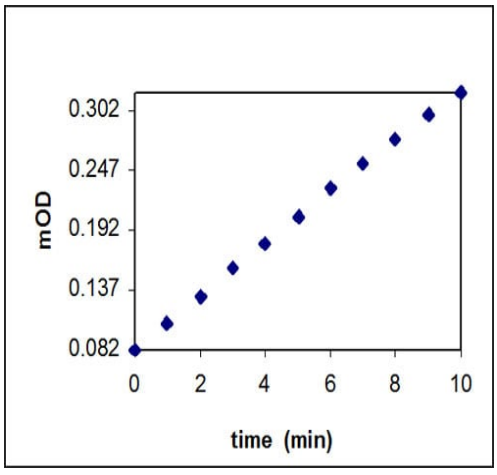
Properties

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
MMP14 Enzyme (Human, Recombinant)	1 x 25µl

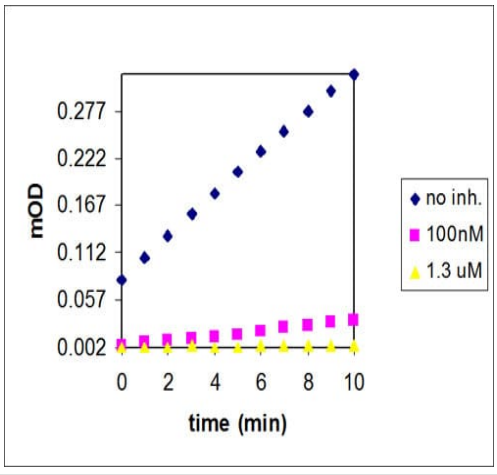
Function	Seems to specifically activate progelatinase A. May thus trigger invasion by tumor cells by activating progelatinase A on the tumor cell surface. May be involved in actin cytoskeleton reorganization by cleaving PTK7.
Tissue specificity	Expressed in stromal cells of colon, breast, and head and neck. Expressed in lung tumors.
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.
Post-translational modifications	The precursor is cleaved by a furin endopeptidase.
Cellular localization	Membrane. Melanosome. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

Images

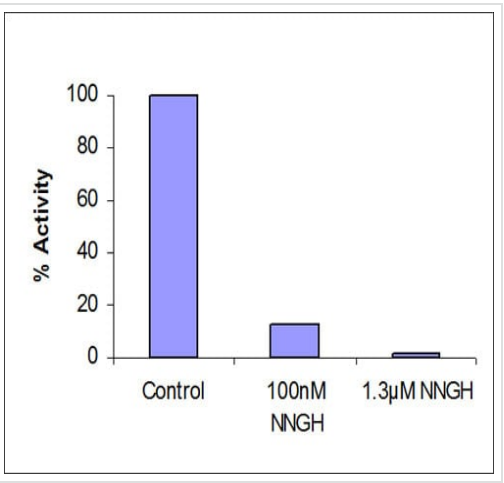


Plot of OD vs. time

Slope=V=2.42E-02 OD/min



Inhibition of MMP14 by NNGH. Example of inhibitor data.



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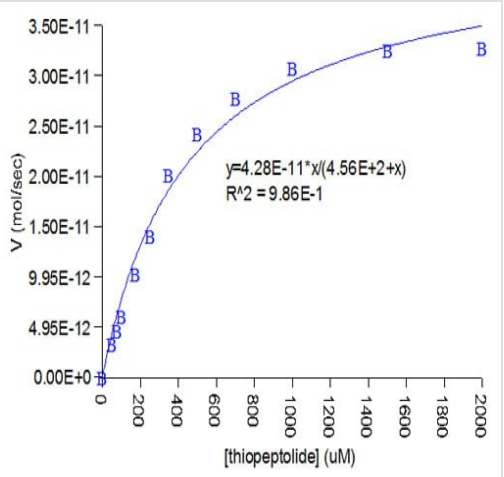

Example graph for Km and Vmax determination.

Km=456 µM

Vmax=42.8 pmol/sec

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