

MMP12 Inhibitor Screening Assay Kit (Colorimetric) ab139441

画像数 4

製品の概要

製品名	MMP12 Inhibitor Screening Assay Kit (Colorimetric)
検出方法	Colorimetric
サンプルの種類	Inhibitor compounds
アッセイタイプ	Enzyme activity
製品の概要	Abcam's MMP12 Inhibitor Screening Assay Kit (Colorimetric) (ab139441) is a complete assay system designed to screen MMP12 inhibitors using a thiopeptide as a chromogenic substrate (Ac-PLG-[2-mercapto-4-methyl-pentanoyl]-LG-OC ₂ H 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.
特記事項	<p>This kit is useful to screen inhibitors of MMP12, a potential therapeutic target. The MMP inhibitor NNGH is also included as a prototypic control inhibitor.</p> <p>Thiol inhibitors should not be used with this kit, as they may interfere with the colorimetric assay.</p>
試験プラットフォーム	Microplate reader

製品の特性

保存方法 Please refer to protocols.

内容	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
MMP12 Enzyme (Human, Recombinant)	1 x 14μl

機能 May be involved in tissue injury and remodeling. Has significant elastolytic activity. Can accept

large and small amino acids at the P1' site, but has a preference for leucine. Aromatic or hydrophobic residues are preferred at the P1 site, with small hydrophobic residues (preferably alanine) occupying P3.

組織特異性

Found in alveolar macrophages but not in peripheral blood monocytes.

配列類似性

Belongs to the peptidase M10A family.

Contains 4 hemopexin-like domains.

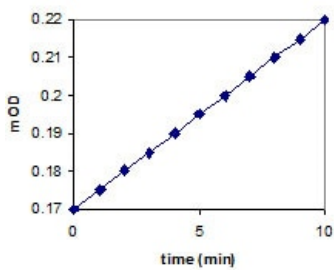
ドメイン

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.

細胞内局在

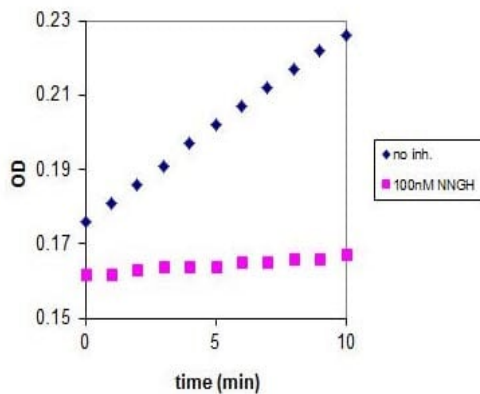
Secreted > extracellular space > extracellular matrix.

画像



Slope= $V=4.85\text{E-}03$ OD/min.

Plot of OD vs. time.

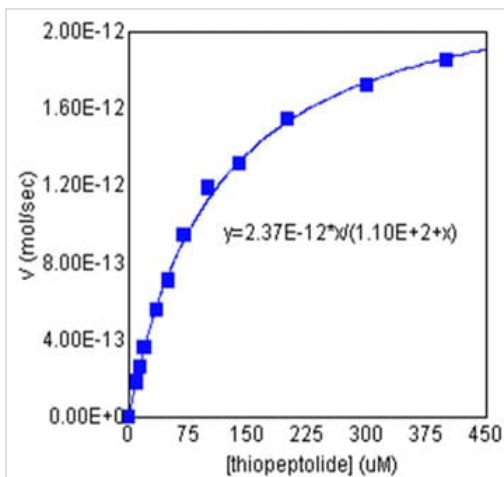


control slope = $5.08\text{E-}03$ OD/min

inhibitor slope = $4.82\text{E-}04$ OD/min

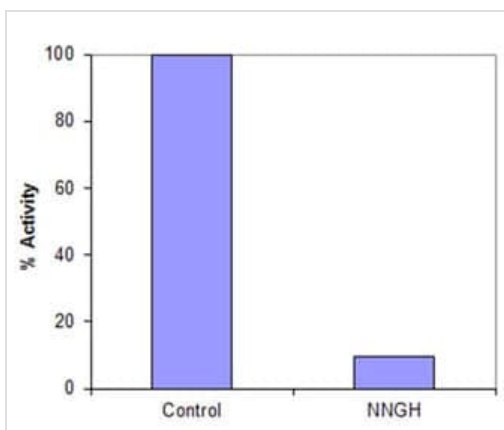
inhibitor % activity remaining = $(4.82\text{E-}04/5.08\text{E-}03) \times 100 = 9.49\%$

Inhibition of MMP12 by NNGH (100 nM). Example of inhibitor data.



Example graph for Km and Vmax determination

Activity of a control sample =
 $(4.85\text{E-}03\text{OD/min} \times 1\text{E-}04\text{L}) / (13,600\text{M-}1\text{cm-}1 \times 0.5\text{cm}) =$
 $7.13\text{E-}11 \text{ mol/min at } 37^\circ\text{C, } 100\text{?M thiopeptolide}$



Inhibition of MMP12 by NNGH (100 nM).

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