MMP13 Inhibitor Screening Assay Kit (Colorimetric) ab139450

Overview

Product name: MMP13 Inhibitor Screening Assay Kit (Colorimetric)
Detection method: Colorimetric
Sample type: Inhibitor compounds
Assay type: Enzyme activity

Product overview: Abcam MMP13 Inhibitor Screening Assay Kit (Colorimetric) (ab139450) is a complete assay system designed to screen MMP13 inhibitors using a thiopeptide as a chromogenic substrate (Ac-PLG-[2-mercapto-4-methyl-pentanoyl]-LG-OC₂H₅). 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 MMP13, 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.

<table>
<thead>
<tr>
<th>Components</th>
<th>1 x 96 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-well Clear Microplate 1/2 Volume</td>
<td>1 unit</td>
</tr>
<tr>
<td>Colorimetric Assay Buffer</td>
<td>1 x 20ml</td>
</tr>
<tr>
<td>MMP Inhibitor</td>
<td>1 x 50µl</td>
</tr>
<tr>
<td>MMP Substrate</td>
<td>1 x 50µl</td>
</tr>
<tr>
<td>MMP13 Enzyme (Human, Recombinant)</td>
<td>1 x 53µl</td>
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</table>
**Function**
Degrades collagen type I. Does not act on gelatin or casein. Could have a role in tumoral process.

**Tissue specificity**
Seems to be specific to breast carcinomas.

**Involvement in disease**
Defects in MMP13 are the cause of spondyloepimetaphyseal dysplasia Missouri type (SEMD-MO) [MIM:602111]. A bone disease characterized by moderate to severe metaphyseal changes, mild epiphyseal involvement, rhizomelic shortening of the lower limbs with bowing of the femora and/or tibiae, coxa vara, genu varum and pear-shaped vertebrae in childhood. Epimeta physeal changes improve with age.

Defects in MMP13 are the cause of metaphyseal anadysplasia type 1 (MANDP1) [MIM:602111]. Metaphyseal anadysplasia consists of an abnormal bone development characterized by severe skeletal changes that, in contrast with the progressive course of most other skeletal dysplasias, resolve spontaneously with age. Clinical characteristics are evident from the first months of life and include slight shortness of stature and a mild varus deformity of the legs. Patients attain a normal stature in adolescence and show improvement or complete resolution of varus deformity of the legs and rhizomelic micromelia.

**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=$V=7.00\times10^{-3}$ OD/min

**Inhibition of MMP13 by NNGH**
control slope = $7.00\times10^{-3}$ OD/min
inhibitor (100nM) slope = $4.50\times10^{-4}$ OD/min
inhibitor % activity remaining = $(4.50\times10^{-4} / 7.00\times10^{-3}) \times 100 = 6.4\%$
Inhibition of MMP13 by NNGH

Example graph for Km and Vmax determination

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