CREATIVE BIOMART®
Assay Kit

MMP2 Inhibitor Screening Assay Kit

Product Information

Cat

Kit-0320C

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Product Overview

The MMP-2 Colorimetric Drug Discovery Kit is a complete assay system designed to screen MMP-2 inhibitors using a thiopeptide as a chromogenic substrate (AcPLG-[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-1cm-1 at pH 6.0 and above). The assays are performed in a convenient 96-well microplate format. The kit is useful to screen inhibitors of MMP-2, a potential therapeutic target. An inhibitor, NNGH12, 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.

Description

Matrix metalloproteinase-2 (MMP-2, gelatinase A, 72 kDa type IV collagenase) is a member of the MMP family of extracellular proteases. These enzymes play a role in many normal and disease states by virtue of their broad substrate specificities. Targets of MMP-2 include native and denatured collagens, fibronectin, elastin, laminin-5, pro-TNF-a, and neurocan. MMP-2 is secreted as a 72 kDa proenzyme (as measured by SDS-PAGE), and activated by cleavage to 62 and 59 kDa. MMP-2 is an important target for inhibitor screening due to its involvement in diseases such as atherosclerosis, and cancer growth, angiogenesis, and metastasis.

Storage

Store all components except the microplate (room temperature) at -70°C for the highest stability. The MMP-2 enzyme should be handled carefully in order to retain maximal enzymatic activity. It is stable, in diluted or concentrated form, for several hours on ice. As supplied, MMP-2 enzyme is stable for at least 5 freeze/thaw cycles. To minimize the number of freeze/thaw cycles, aliquot the

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MMP-2 into separate tubes and store at -70°C. When setting up the assay, do not maintain diluted components at reaction temperature (e.g. 37°C) for an extended period of time prior to running the assay.

Shipping

Dry Ice

Size

96 tests

Kit Components

96-well Clear Microplate (1/2 Volume), Room temperature
Colorimetric Assay Buffer 1 x 20ml, FORM: 50mM HEPES, 10mM CaCl2, 0.05% Brij-35, 1mM DTNB, pH7.5, STORAGE: -20°C
MMP Inhibitor 1 x 50µl, FORM: 1.3 mM in DMSO, STORAGE: -70°C
MMP Substrate 1 x 50µl, FORM: 25 mM (16.4 mg/ml) in DMSO, STORAGE: -20°C
MMP2 Enzyme (Human, Recombinant) 1 x 45.7µl, FORM: P. pastoris recombinant human MMP-2 catalytic domain (calculated MW 40 kDa), One U=100 pmol/min@ 37°C, 100 µM thiopeptide P125. STORAGE: -70°C; Avoid freeze/thaw cycles.

Materials Required but Not Supplied

Microplate reader capable of measuring A412 to ≥3-decimal accuracy.
Pipetman or multi-channel pipetman capable of pipetting 10-100 µl accurately
(note: reagents can be diluted to increase the minimal pipetting volume to >10 µl).
Ice bucket to keep reagents cold until use.
Water bath or incubator for component temperature equilibration.

Assay Protocol

1. Briefly warm kit components MMP Substrate and MMP Inhibitor to RT to thaw DMSO.
2. Dilute MMP Inhibitor (NNGH) 1/200 in Assay Buffer as follows. Add 1 µL inhibitor into 200 µL Assay Buffer, in a separate tube. Warm to reaction temperature (e.g. 37°C).
3. Dilute MMP2 substrate 1/25 in assay buffer to required total volume (10 µL are needed per well). For example, for 15 wells dilute 6.4 µL MMP substrate into 153.6 µL assay buffer, in a separate tube.

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Warm to reaction temperature (e.g. 37°C).
4. Dilute MMP2 enzyme 1/56 in assay buffer to required total volume (20 µL are needed per well). Warm to reaction temperature (e.g. 37°C) shortly before assay.
5. Pipette assay buffer into each desired well of the 1/2 volume microplate as follows:
Blank (no MMP-9)=90 µL Assay Buffer
Control (no inhibitor)=70 µL Assay Buffer
MMP Inhibitor=50 µL Assay Buffer
Test inhibitor=varies (see Table 1, below)
6. Allow microplate to equilibrate to assay temperature (e.g. 37°C).
7. Add 20 µL MMP2 Enzyme (diluted in step 4) to the control, MMP Inhibitor, and test inhibitor wells. Final amount of MMP2 will be 1.16 U per well (11.6 mU/µL). Remember to not add MMP2 to the blanks!
8. Add 20 µL MMP inhibitor (diluted in step 2) to the MMP Inhibitor wells only! Final inhibitor concentration=1.3 µM.
Note: 1.3 µM NNGH will inhibit MMP2 by approximately 94% under these conditions.
9. Add desired volume of test inhibitor to appropriate wells. See Table 1, below.
10. Incubate plate for 30-60 minutes at reaction temperature (e.g. 37°C) to allow inhibitor/enzyme interaction.
11. Start reaction by the addition of 10 µL MMP Substrate (diluted and equilibrated to reaction temperature in step 3). Final substrate concentration=100 µM.
12. Continuously read plates at A412nm in a microplate reader. Record data at 1 min. time intervals for 10 to 20 min.
13. Perform data analysis (see below).
NOTE: Retain microplate for future use of unused wells.
TABLE 1. Example of Samples.

Sample	Assay buffer	MMP-2 Inhibitor (58 mU/ul)	Substrate (6.5 µM)	Total Volume (1 mM)
Blank	90 µl	0	0	10 µl 100 µl
Control	70 µl	20 µl	0	10 µl 100 µl
Inhibitor NNGH	50 µl	20 µl	20 µl	10 µl 100 µl

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Test inhibitor* X µl 20 µl Y µl 10 µl 100 µl
*Test inhibitor is the experimental inhibitor. Dissolve/dilute inhibitor into assay buffer and add to appropriate wells at desired volume "Y". Adjust volume "X" to bring the total volume to 100 µl.

Example of plate: well# sample
A1 Blank
B1 Blank
C1 Control
D1 Control
E1 Inhibitor NNGH
F1 Inhibitor NNGH
G1 Test inhibitor
H1 Test inhibitor

Analysis

Plotting
1. Plot data as OD versus time for each sample.
2. Determine the range of time points during which the reaction is linear. Typically, points from 1 to 10 min are sufficient.
3. Obtain the reaction velocity (V) in OD/min: determine the slope of a line fit to the linear portion of the data plot using an appropriate routine.
4. Average the slopes of duplicate samples.
Data Reduction
5. If the blank has a significant slope, subtract this number from all samples.
6. To determine inhibitor % remaining activity:
Inhibitor % activity remaining = (V inhibitor / V control) x 100
7. To find the activity of the samples expressed as mol substrate/min, employ the following equation:
X mol substrate/min=(V x vol.)/(ε x l)
Where V is reaction velocity in OD/min, vol. is the reaction volume in liters, ε is the extinction coefficient of the reaction product (2-nitro-5-thiobenzoic acid)(13,600 M1 cm-1), and l is the path length of light through the sample in cm (for 100 ul in the supplied microplate, l is 0.5 cm).

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Note: The above equation determines enzyme activity in terms of moles of thiopeptolide substrate P125 converted per minute. Under these conditions, the secondary substrate DTNB is saturating, and the velocity of DTNB conversion to 2-nitro-5-thiobenzoic acid is not rate-limiting.

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