CREATIVE BIOMART®
Assay Kit

Matrix Metalloproteinase-10 (MMP-10) fluorometric drug discovery Kit

Product Information

Cat.No. Kit-0569

Product Overview

The MMP-10 Fluorometric Drug Discovery Kit is a complete assay system designed to screen MMP-10 inhibitors using a quenched fluorogenic peptide: fluorogenic substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH2 [Mca=(7- methoxycoumarin-4-yl)-acetyl; Dpa=N-3-(2,4-dinitrophenyl)-L- a-β-diaminopropionyl]. Mca fluorescence is quenched by the Dpa group until cleavage by MMPs at the Gly-Leu bond separates the two moieties. The assays are performed in a convenient 96-well microplate format. The kit is useful to screen inhibitors of MMP-10, a potential therapeutic target. The compound NNGH11 is also included as a prototypic control inhibitor.

Storage

at -80°C

Shipping

Dry Ice

Size

96 wells

Kit Components

MMP-10 ENZYME (HUMAN, RECOMBINANT)
FORM: E. coli recombinant human MMP-10 catalytic domain (calculated MW 19.4 kDa), 5 U/µl.
UNIT DEFINITION: One unit is defined as the amount of enzyme that will hydrolyze 100uM thiopeptide Ac-PLG-[2-mercapto-4-methyl-pentanoyl]-LG-OC2H5 at 100 pmol/min at 37°C.
STORAGE: -70°C; Avoid freeze/thaw cycles
QUANTITY: 150 U
PRESENTATION: 30 µl in screw-cap microfuge vial.

SUBSTRATE (fluorogenic substrate peptide; MW=1093.2)
FORM: 400 uM (437 ug/ml) in DMSO (dimethylsulfoxide)

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STORAGE: -70°C
PRESENTATION: 200 ul in amber screw-cap microfuge vial.

CALIBRATION STANDARD (fluorogenic control peptide, MCA-Pro-Leu-OH; MW=444.5)
FORM: 40 uM (17.8 ug/ml) in DMSO
STORAGE: -70°C
PRESENTATION: 50 ul in amber screw-cap microfuge vial.

INHIBITOR (NNGH; MW=316.4)
FORM: 1.3mM in DMSO
STORAGE: -20 or -70°C
PRESENTATION: 50ul in screw-cap microfuge vial.

ASSAY BUFFER
50mM HEPES, 10mM CaCl2, 0.05% Brij-35, pH 7.5
FORM: Liquid in screw-cap plastic bottle
STORAGE: Room temperature
QUANTITY: 20 ml

½ VOLUME 96-WELL WHITE NBS MICROPLATE
STORAGE: Room temperature.

Materials Required but Not Supplied

Fluorescent microplate reader capable of excitation at 328nm and emission at 420nm. The following Ex/Em have also been used: 320,340/393,400,405.
Pipetmen or multi-channel pipetmen capable of pipetting 1-100 µl accurately.
Ice bucket to keep reagents cold until use.
Water bath or incubator for component temperature equilibration.

Technical Notes

Note on storage: Store all components except the microplate and assay buffer (room temperature)

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at -70°C for the highest stability. The MMP-10 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-10 enzyme is stable for at least 5 freeze/thaw cycles. To minimize the number of freeze/thaw cycles, aliquot the MMP-10 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

Assay Protocol

1. Briefly warm kit components SUBSTRATE, CALIBRATION STANDARD, and INHIBITOR to RT to thaw DMSO.
2. Dilute 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. Thaw the DMSO stock vial of substrate and dilute sufficient volume to 40µM in assay buffer (10ul needed per well). Warm to reaction temperature (e.g. 37°C).
4. Dilute MMP-10 enzyme 1/100 in assay buffer to required total volume (20 µl are needed per well). Warm to reaction temperature (e.g. 37°C).
5. Pipet assay buffer into each desired well of the 1/2 volume microplate as follows:
Calibration = 80 ul in 3 wells (see step 11)
Control (no inhibitor) = 70 µl
Inhibitor NNGH = 50 µl
Test inhibitor = varies (see Table 1)
Example of plate:
well# sample
A1 Calibration
B1 Calibration
C1 Calibration
D1 Control
E1 Control
F1 Inhibitor NNGH
G1 Inhibitor NNGH
H1 Test inhibitor

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A2… Test inhibitor…
6. Allow microplate to equilibrate to assay temperature (e.g. 37°C).
7. Add 20 µl MMP-10 (diluted in step 4) to the control, inhibitor NNGH, and test inhibitor wells. Final amount of MMP-10 will be 1 U per well (10.0 mU/ul). Remember to not add MMP-10 to the calibration wells!
8. Add 20 µl NNGH inhibitor (diluted in step 2) to the inhibitor NNGH wells only. Final inhibitor concentration = 1.3µM.
9. Add desired volume of test inhibitor to appropriate wells. See Table 1.
10.Incubate plate for 30-60 minutes at reaction temperature (e.g. 37°C) to allow inhibitor/enzyme interaction.
11.In the meantime, calibrate the fluorescent microplate reader, using Ex/Em=328/420: Prewarm assay buffer to reaction temperature in 3 wells in the microplate, then to each add 10µl substrate peptide to give the concentration to be used in the assay (e.g., for 4 uM final add 10µl 40µM) and mix. When the fluorescent signal is constant, use this reading as the zero (Blank) value in arbitrary fluorescence units (RFUs). Using the same wells, with their mixtures of substrate peptide and buffer, add 10µl calibration standard peptide to give 3 different final molar concentrations ranging between 2 and 10% of the substrate peptide molar concentration (e.g., 80, 200, and 400 nM) and measure their fluorescence. Use these values to build a standard curve relating micromolar CALIBRATION STANDARD concentration (x axis) to RFUs (y axis). The slope of the line is the conversion factor (CF). If multiple concentrations of substrate peptide are used, such as in kinetic determinations, step 11 must be performed for each concentration, due to absorptive quenching by the substrate peptide. Note: this calibration can be done at any time.
12.Start reactions by the addition of 10 µl substrate (diluted and equilibrated to reaction temperature in step 3). Final substrate concentration = 4 µM.
13.Continuously read plates in the fluorescent microplate reader, using Ex/Em=328/420. For example, record data at 1 minute time intervals for 10 minutes.
14.Perform data analysis (see below).
NOTE: Retain microplate for future use of unused wells.
TABLE 1. Example of Samples.
Sample Assay buffer MMP-10 (50 mU/ul) Inhibitor (6.5 µM) Substrate (40µM) Total Volume

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Control 70 µl 20 µl 0 10 µl 100 µl
Inhibitor NNGH 50 µl 20 µl 20 µl 10 µl 100 µl
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.
15. Plot data as RFUs (minus Blank RFU value determined during calibration, step 11) versus time for each sample.
16. Determine the range of initial time points during which the reaction is linear.
17. Obtain the initial reaction velocity (V) in RFUs/min: determine the slope of a line fit to the initial linear portion of the data plot using an appropriate routine.
18. It is best to use a range of inhibitor concentrations, each in duplicate. Average the slopes of duplicate samples.

Analysis

To determine inhibitor % remaining activity:
Inhibitor % activity remaining = (V inhibitor / V control) x 100

To determine the activity of the samples expressed as picomoles substrate hydrolyzed per minute:
X pmoles substrate/min = 1/CF x V x vol
Where: CF is the conversion factor (micromolar concentration/RFUs, from step 11), V is initial reaction velocity (RFUs/min, from step 17), and vol is the reaction volume in microliters (100).