Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

MMP-2 Inhibitor Screening Kit (Fluorometric)

rev 06/21

(Catalog # K2017-100; 100 assays; Store at -20 °C)

I. Introduction:

Matrix metalloproteinase-2 (MMP-2) is a zinc-dependent endopeptidase family member that is capable of degrading denatured collagen, collagen type IV as well as other extracellular matrix proteins in normal physiological processes such as embryonic development, tissue remodeling and in disease processes including metastasis, atherosclerosis, cardiac dysfunction etc. MMP-2 is widely expressed in almost all cell types. Like most MMP's, MMP-2 is secreted as inactive pro-protein that is activated when cleaved by extracellular proteases. Additionally, MMP-2 can be activated intracellularly via S-Glutathionylation. **BioVision's MMP-2 Inhibitor Screening Kit** provides a quick and sensitive way for screening, studying and characterizing potential inhibitors of MMP-2. In this assay, MMP-2 hydrolyzes a FRET-based MMP-2 substrate and releases the quenched fluorescent group, Mca, which can then be detected fluorometrically at Ex/Em = 325/393 nm. A potent, specific MMP-2 inhibitor is also included in the kit.

FRET based MMP-2 Substrate $\xrightarrow{\textbf{MMP-2}}$ Mca (Ex/Em = 325/393 nm)

II. Application:

- Screening or characterizing MMP-2 inhibitors.

III. Kit Contents:

Components	K2017-100	Cap Code	Part Number
MMP-2 Assay Buffer	25 ml	WM	K2017-100-1
MMP-2 Substrate	100 µl	Red	K2017-100-2
Recombinant MMP-2	1 vial	Blue	K2017-100-3
Inhibitor (NNGH, 2 mM)	50 µl	Yellow	K2017-100-4

IV. User Supplied Reagents and Equipment:

- 30% Glycerol solution
- 96-well white plate with flat bottom (low/medium binding)
- Multi-well spectrophotometer (Fluorescent plate reader)

V. Storage Conditions and Reagent Preparation:

Store kit at -20 °C, protected from light. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.

- **MMP-2 Assay Buffer & MMP-2 Substrate:** Warm to room temperature (RT) before use.
- **Recombinant MMP-2:** Reconstitute with 110 µl pre-chilled 30% Glycerol solution (in dH_2O). Keep on ice until it completely dissolves. Aliquot and store the reconstituted MMP-2 stock solution at -20 °C. Avoid repeated freeze/thaw cycles.
- **Inhibitor (NNGH, 2 mM):** Ready to use. Warm to room temperature (RT) before use. Aliquot and store at -20 °C.

VI. MMP-2 Inhibitor Screening Protocol:

1. Pre-activate Recombinant MMP-2: Dilute the reconstituted Recombinant MMP-2, 50 fold by mixing 10 µl of reconstituted MMP-2 stock solution with 490 µl of MMP-2 Assay Buffer. Mix thoroughly and keep on ice. Add 50 µl of the diluted MMP-2 enzyme into desired wells of a 96-well white plate labeled as Sample, Solvent Control, Inhibitor Control and Enzyme Control respectively. **Incubate at 37 °C for 30 min to activate the enzyme.**

2. Screening Test Inhibitor(s): Dissolve Test Inhibitor(s) in an appropriate solvent to make 100X stock solution. Dilute the stock Test Inhibitor to 4X using MMP-2 Assay Buffer. Add 25 µl of diluted Test Inhibitor into the Sample (S) well(s). Add 25 µl of 4X Solvent (4X final well solvent concentration) into the Solvent Control well.

Note: Solvents used to solubilize the Test Inhibitor(s) might affect the enzymatic activity. Prepare a Solvent Control well with the same final concentration of solvent used to dissolve the Test Inhibitor(s).

3. Enzyme, Background and Inhibitor Control Preparation: Add 25 µl of MMP-2 Assay Buffer to the Enzyme Control (EC) well. For Background Control (BC), add 75 µl of MMP-2 Assay Buffer in a separate empty well. Add 2 µl of Inhibitor **(NNGH)** to the Inhibitor Control (IC) well and bring up the volume to 75 µl/well using MMP-2 Assay Buffer. ***IC_{50} estimation (Optional):*** Prepare several dilutions of the Test Inhibitor(s) in MMP-2 Assay Buffer maintaining consistent final Solvent Concentration in all wells. Add 25 µl of each dilution into the designated wells. At this stage, all wells including Sample, Solvent Control, Inhibitor Control, Enzyme Control and Background Control contain 75 µl.

4. Reaction Mix Preparation: Mix enough reagents for the number of assays to be performed. For each well, prepare 25 µl Mix Reaction Mix containing

	Reaction Mix
MMP-2 Assay Buffer	24 µl
MMP-2 Substrate	1 µl

Add 25 µl Reaction Mix to Sample, Solvent Control, Inhibitor Control, Enzyme Control and Background Control wells. The total reaction volume is 100 µl/well.

5. Measurement: Measure fluorescence in a kinetic mode at Ex/Em = 325/393 nm at 1 min intervals for 30-60 min at 37 °C.

6. Calculation: Obtain ΔRFU for all Test Inhibitors, Enzyme Control, Solvent Control and Inhibitor Control by subtracting RFU at time t_1 from RFU at time t_2, such that t_2 and t_1 is within a linear range of the assay. Calculate the slope for all Samples including Enzyme Control **[EC]** by dividing ΔRFU by time Δt ($t_2 - t_1$). If Solvent Control **[SC]** slope is significantly different from Enzyme Control **[EC]** slope, use its values instead of EC in the calculations shown below.

$$\textbf{\% Relative Inhibition} = \frac{\textbf{Slope of}[\ \textbf{EC}] - \textbf{Slope of}\ [\textbf{S}]}{\textbf{Slope of}[\textbf{EC}]} \textbf{X100}$$

Note: Subtract the reading of Background Control [BC] from all Test Samples [S], Enzyme Control [EC], and Solvent Control [SC].

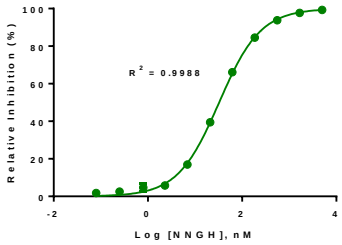

Figure: Inhibition MMP-2 activity by NNGH, a MMP inhibitor (BV Cat# 2569). IC_{50} of NNGH was calculated to be 32.5 nM. Assay was performed following the kit protocol.

VII. Related Products:

MMP-2, Human CellExp™, human recombinant (Cat. # 7245)
MMP-2 Catalytic Domain Recombinant Human (Cat. # 7782)
Pro-Matrix Metalloproteinase-2 (Pro-MMP-2) (Cat. # 7780)
MMP-2 Polyclonal Antibody (Cat. # 5562)
MMP-2 Polyclonal Antibody (Cat. # 5562R)
MMP-2 (Human) ELISA Kit (Cat. # E4723-100)
MMP-2 (Rat) ELISA Kit (Cat. # E4724-100)
MMP-2 (Mouse) ELISA Kit (Cat. # E4725-100)
MMP-9, Active, human recombinant (Cat. # 7867)
MMP-14, Active, human recombinant (Cat. # 8009)
MMP-3 Activity Fluorometric Assay Kit (Cat. # K783-100)

MMP-1 Inhibitor Screening Kit (Fluorometric) (Cat. # K794-100)
MMP-3 Inhibitor Screening Kit (Fluorometric) (Cat. # K793-100)
MMP-9 Inhibitor Screening Kit (Fluorometric) (Cat. # K844-100)
MMP-14 Inhibitor Screening Kit (Fluorometric) (Cat. # K846-100)
MMP-3 Activity Fluorometric Assay Kit (Cat. # K783-100)
MMP-III Inhibitor, NNGH (Cat. # 2569)
MMP-1, Human CellExp™, human recombinant (Cat. # 7244)
MMP-3, Human CellExp™, human recombinant (Cat. # P1310)
MMP-9, Human CellExp™, human recombinant (Cat. # 7246)
Recombinant Human MMP-1 Catalytic Domain (Cat. # 7781)
Recombinant Human MMP-13 Catalytic Domain (Cat. # P1230)

FOR RESEARCH USE ONLY! Not to be used on humans.