

MMP-13 Inhibitor Screening Kit (Fluorometric)

12/20

(Catalog # K2067-100; 100 assays; Store at -20 °C)

I. Introduction:

Matrix metalloproteinase-13 (MMP-13; Collagenase-3) is a zinc-dependent endopeptidase family member that is capable of degrading many protein components of the extracellular matrix (ECM) including type II collagen in articular cartilage and bone. It is produced and secreted as a 60-kDa inactive, precursor form (proMMP-13), which is activated to a 48-kDa form by extracellular proteinases such as plasmin, MMP-2, MMP-3 and MMP-14. MMP-13 has wide substrate specificity, as it degrades collagen types (I-IV) but preferentially hydrolyzes type II collagen. Additionally, it cleaves other ECM molecules including fibronectin, aggrecan, osteonectin etc. MMP-13 plays a major role in ECM remodeling during fetal bone development, post-natal bone remodeling, tissue remodeling etc. It is overexpressed in numerous pathological conditions including rheumatoid arthritis, osteoarthritis, atherosclerosis, aortic aneurysms, cancer etc. Thus, developing and screening of selective MMP-13 inhibitors is under intensive investigation for therapeutic intervention. **BioVision's MMP-13 Inhibitor Screening Assay Kit** provides a quick, easy and sensitive assay for screening, studying and characterizing potential inhibitors of MMP-13. In this assay, MMP-13 hydrolyzes a FRET-based MMP-13 substrate and releases unquenched by the catalysis of fluorescent group, Mca, which is detected fluorometrically at Ex/Em = 325/393 nm. A potent, specific MMP-13 inhibitor is also included in the kit.



II. Application:

- Screening or characterizing MMP-13 inhibitors.

III. Kit Contents:

Components	K2067-100	Cap Code	Part Number
MMP-13 Assay Buffer	25 ml	WM	K2067-100-1
MMP-13 Substrate	100 µl	Red	K2067-100-2
Recombinant MMP-13	25 µl	Blue	K2067-100-3
MMP-13 Inhibitor (2 mM)	50 µl	Yellow	K2067-100-4

IV. User Supplied Reagents and Equipment:

- DMSO
- 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-13 Assay Buffer:** Warm to room temperature (RT) before use.
- **MMP-13 Substrate:** Warm to RT before use. Divide into aliquots and store at -20 °C, protected from light.
- **Recombinant MMP-13:** Thaw on ice. Divide into aliquots and store at -20 °C. Avoid repeated freeze/thaw cycles.
- **MMP-13 Inhibitor (2 mM):** Warm to RT. Divide into aliquots and store at -20 °C. Prepare 1:10 dilution of the 2 mM MMP-13 Inhibitor in DMSO (not provided) to make 200 µM MMP-13 Inhibitor. Diluted MMP-13 Inhibitor can be aliquoted and stored at -20 °C. Avoid repeated freeze/thaw cycles.

VI. MMP-13 Inhibitor Screening Protocol:

1. Recombinant MMP-13: Dilute Recombinant MMP-13, at a 1:125 dilution with MMP-13 Assay Buffer (i.e. for 10 reactions, dilute 2 µl MMP-13 with 248 µl of MMP-13 Assay Buffer). Mix thoroughly and keep on ice. Add 25 µl of the diluted MMP-13 enzyme into the desired wells of a 96-well white plate labeled as **Sample**, **Solvent Control**, **Inhibitor Control** and **Enzyme Control** respectively.

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-13 Assay Buffer. Add 25 µl of diluted Test Inhibitor into the Sample 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. Thus, prepare a Solvent Control well with the same final concentration of solvent used to dissolve the Test Inhibitor(s).

3. Enzyme Control, Background Control and Inhibitor Control Preparation: Add 25 µl of MMP-13 Assay Buffer to the **Enzyme Control** well. For **Background Control (BC)**, add 50 µl of MMP-13 Assay Buffer in a separate well. To the **Inhibitor Control** well, add 2 µl of diluted 1:10 MMP-13 Inhibitor and adjust the volume to 50 µl/well by adding 23 µl MMP-13 Assay Buffer. At this stage, all wells including Sample, Solvent Control, Inhibitor Control, Enzyme Control and Background Control contain 50 µl/well. Incubate for 10 minutes at RT.

IC₅₀ estimation (Optional): Prepare several dilutions of the Test Inhibitor(s) in MMP-13 Assay Buffer while maintaining the consistent final Solvent Concentration in all wells. Add 25 µl of each dilution into the designated wells.

4. MMP-13 Substrate Mix Preparation: Mix enough Substrate Mix for the number of assays to be performed. Prepare 50 µl Substrate Mix per reaction.

	<u>Substrate Mix</u>
MMP-13 Assay Buffer	49 µl
MMP-13 Substrate	1 µl



Add 50 µl Substrate 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 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 Samples [S], Enzyme Control [EC], Solvent Control [SC] and Inhibitor Control [IC] by subtracting RFU at time t₁ from RFU at time t₂, such that t₂ and t₁ is within a linear range of the assay. Calculate the slope for all Samples including Enzyme Control [EC] by dividing ΔRFU by time Δt (t₂ - t₁). If [SC] slope is significantly different from [EC] slope, use its values instead of EC in the calculations shown below.

$$\% \text{ Relative Inhibition} = \frac{\text{Slope of [EC]} - \text{Slope of [S]}}{\text{Slope of [EC]}} \times 100$$

Note: Subtract the reading of [BC] wells from all [S], [EC], and [SC] wells.

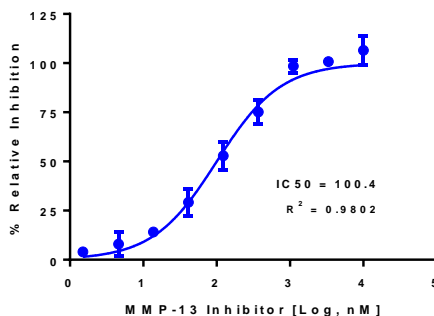


Figure: Inhibition of MMP-13 activity by the MMP-13 inhibitor (BioVision Cat. # 9607). IC₅₀ was calculated to be 100.4 nM. Assay was performed following the kit protocol.

VII. Related Products:

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| Recombinant Human MMP-13 Catalytic Domain (Cat. # P1230) | MMP-1 Inhibitor Screening Kit (Fluorometric) (Cat. # K794-100) |
| Human CellExp™ MMP-13, Human Recombinant (Cat. # P1566) | MMP-2 Inhibitor Screening Kit (Fluorometric) (Cat. # K2017-100) |
| Pro-Matrix Metalloproteinase 13 (Pro-MMP-13) (Cat. # 7785) | MMP-3 Inhibitor Screening Kit (Fluorometric) (Cat. # K793-100) |
| MMP-1, Human CellExp™, human recombinant (Cat. # 7244) | MMP-9 Inhibitor Screening Kit (Fluorometric) (Cat. # K844-100) |
| Recombinant Human MMP-1 Catalytic Domain (Cat. # 7781) | MMP-14 Inhibitor Screening Kit (Fluorometric) (Cat. # K846-100) |
| MMP-2, Human CellExp™, human recombinant (Cat. # 7245) | MMP-3 Activity Fluorometric Assay Kit (Cat. # K783-100) |
| MMP-2 Catalytic Domain Recombinant Human (Cat. # 7782) | MMP-III Inhibitor, NNGH (Cat. # 2569) |
| Pro-Matrix Metalloproteinase-2 (Pro-MMP-2) (Cat. # 7780) | MMP-13 Inhibitor (Cat. # 2392) |
| Recombinant Human MMP-3 Catalytic Domain (Cat. # 7783) | MMP-9 Inhibitor (Cat. # 1981) |
| MMP-3, Human CellExp™, human recombinant (Cat. # P1310) | Batimastat (Cat. # 1704) |

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