

MMP-14 Inhibitor Screening Kit (Fluorometric)

9/15

(Catalog # K846-100; 100 assays; Store at -20°C)

I. Introduction:

The Matrix metalloproteinase-14 (MMP-14) (MT1-MMP) belongs to a family of zinc-dependent endopeptidases that are responsible for the degradation of the extracellular matrix (ECM) proteins including collagens, elastins, gelatin, matrix glycoproteins and proteoglycans, during normal development and disease processes. MMPs can be classified into at least four different families of closely related members - collagenases, gelatinases, stromelysins, and membrane-type MMPs (MT-MMPs). MMP-14 belongs to the family of MT-MMPs and is localized on cell surfaces via its transmembrane domain. MMP-14 is expressed in adult lung, placenta, kidney, ovaries, intestine, prostate and spleen along with certain carcinomas. The ability of MMP-14 to degrade type I collagen, and activate pro-MMP-2 and pro-MMP-9 makes it a key enzyme in many physiological and pathological processes such as angiogenesis and tumor invasion. BioVision's MMP-14 Inhibitor Screening Kit provides a simple, fast and high-throughput adaptable method to screen/study/characterize potential MMP-14 inhibitors. The assay utilizes the ability of MMP-14 to cleave a synthetic MCA-based peptide substrate to release free MCA (7-Methoxycoumarin-4-acetic acid), which can be easily quantified using a fluorometer or fluorescence microplate reader at Ex/Em = 325/420 nm. In the presence of a MMP-14 specific inhibitor, the cleavage of the substrate is reduced/abolished, resulting in decrease or total loss of the MCA fluorescence.

II. Application:

- Screening/studying/characterizing potential MMP-14 inhibitors

III. Kit Contents:

Components	K846-100	Cap Code	Part Number
MMP-14 Assay Buffer	25 ml	WM	K846-100-1
MMP-14 Substrate (4 mM)	100 µl	Orange	K846-100-2
MMP-14 Enzyme	1 Vial	Brown	K846-100-3
MMP-14 Inhibitor (NNGH, 2 mM)	50 µl	Yellow	K846-100-4

IV. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom.
- Multi-well spectrophotometer (fluorescent plate reader)

V. Storage Conditions and Reagent Preparation:

Store the kit at -20°C, protected from light. Once opened, store the kit components as per the respective temperatures mentioned below. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **MMP-14 Assay Buffer:** Bring to room temperature (RT) before use. Store at 4°C or -20°C.
- **MMP-14 Substrate:** Ready to use. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use.
- **MMP-14 Enzyme:** Reconstitute with 110 µl dH₂O. Aliquot and store at -20°C. Freeze/thaw should be limited to 1 time. During use, keep the solution on ice at all times.
- **MMP-14 Inhibitor:** Ready to use. Aliquot and store at -20°C. Dilute to 1:20 with MMP-14 Assay Buffer just before use.

VI. MMP-14 Inhibitor Screening Protocol:

1. **MMP-14 Solution Preparation:** Dilute MMP-14 enzyme 1:50 with MMP-14 Assay Buffer just before use. Make as much as needed for the number of compounds to be tested, enzyme control and if necessary solvent control(s). Add 50 µl of diluted MMP-14 enzyme into desired well(s). Add 50 µl of MMP-14 Assay Buffer to a well as Background Control (BC).

Note: The unused diluted enzyme may be stored at -80°C for a week.

2. **Screening compounds, Inhibitor Control (IC) & Enzyme Control (EC) Preparations:** Dissolve test inhibitors into proper solvent to make a 10X stock solution. Add 10 µl of the test inhibitor stock solution (Sample, S) or MMP-14 Assay Buffer (Enzyme Control, EC) into MMP-14 enzyme containing wells. For Inhibitor Control (IC), add 10 µl diluted MMP-14 inhibitor into MMP-14 enzyme well(s). Incubate at room temperature for 10 min.

Note: Solvents used to solubilize the inhibitors might affect the enzymatic activity. If solvent effect on the enzymatic activity is a concern, prepare a solvent control well(s) (SC) with the same final concentration of the solvent(s) as in the inhibitor sample(s).

3. **MMP-14 Substrate Preparation:** For each well, prepare 40 µl of the substrate solution.

39 µl MMP-14 Assay Buffer

1 µl MMP-14 Substrate

Mix & add 40 µl of MMP-14 Substrate solution into Enzyme Control, Inhibitor Control & sample wells. Mix well.

4. **Measurement:** Measure fluorescence (Ex/Em = 325/420 nm) in a kinetic mode for 1hr at 37°C. Choose two time points (T1 & T2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU1 and RFU2)

5. **Calculation:** Calculate the slope for all Samples (S), including Enzyme Control (EC), by dividing the net ΔRFU (RFU2-RFU1) values with the time ΔT (T2-T1).

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

Note: If the values of the Solvent Control (SC) are significantly different from these of the EC, substitute them for the EC in the equation above.

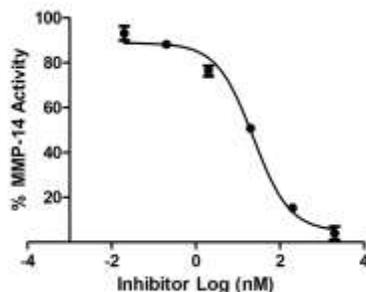


Figure: Inhibition of MMP-14 Enzyme activity by NNGH (Cat # 2569) ($IC_{50} = 20$ nm) Assays were performed following the kit protocol.

VII. RELATED PRODUCTS:

- MMP-1, Human CellExp™, Human Recombinant (Cat # 7244)
- MMP-1 Inhibitor Screening Kit (Fluorometric) (Cat # K794)
- MMP-2, Human Recombinant (Cat # 7782)
- MMP-2, Human CellExp™, human recombinant (Cat # 7245)
- MMP-3 Activity Fluorometric Assay Kit (Cat # K783)
- MMP-3, Human Recombinant (Cat # 7783)
- MMP-9, Active, human recombinant (Cat # 7867)
- MMP-14, Active, Human Recombinant (Cat # 8009)
- MMP-1 Antibody (Cat #5781)
- MMP-2 Antibody (Cat #5562)
- MMP-9 Antibody (Cat #3529, 3969, 5565)
- MMP-11 Antibody (Cat #3531R)
- MMP-17 Antibody (Cat #3537)
- MMP-III Inhibitor, NNGH (Cat #2569)
- MMP-9 inhibitor (Cat #1981)
- GM 6001 (Cat #1799)

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