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MMP-9 Inhibitor Screening Kit (Fluorometric)

rev 9/15

(Catalog # K844; 100 assays; Store at -20°C)

I. Introduction:

Matrix metalloproteinase (MMP) family proteins are involved in the breakdown of extracellular matrix, and have been recognized as potential targets for a variety of pathologies. Matrix metallopeptidase 9 (MMP-9), also known as 92 kDa type IV collagenase, 92 kDa gelatinase or gelatinase B, is the mostly studied MMP, due to its fundamental roles in cancer biology, autoimmune disease, and other conditions. MMP-9 is a metalloenzyme, composed of a pro-domain, a gelatin binding domain, a catalytic domain, a proline rich linker region, and a carboxyl terminal hemopexin like domain. This enzyme degrades various substrates including gelatin, collagen types IV / V, and elastin. BioVision's MMP-9 Inhibitor Screening Kit provides a quick and sensitive way for screening, studying and characterizing potential inhibitors of MMP-9. In this assay, MMP-9 hydrolyzes a FRET-based MMP-9 substrate and releases the quenched fluorescent group Mca, which can be detected fluorometrically at Ex/Em=325/393 nm.

II. Application:

- Screening/studying/characterizing potential MMP-9 inhibitors

III. Kit Contents:

Components	K844-100	Cap Code	Part Number
MMP-9 Assay Buffer	25 ml	WM	K844-100-1
MMP-9 Substrate (4 mM)	100 µl	Orange	K844-100-2
MMP-9 (lyophilized)	1 vial	Green	K844-100-3
Inhibitor (NNGH 2 mM)	50 µl	Yellow	K844-100-4

IV. User Supplied Reagents and Equipment:

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

V. Storage Conditions and Reagent Preparation:

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

- **MMP-9 Assay Buffer and MMP-9 Substrate**: Bring to room temperature before use.
- **MMP-9:** Reconstitute with 110 µl pre-chilled 30% Glycerol solution (in dH_2O). Keep on ice until it completely dissolves. Store the reconstituted MMP-9 in aliquots at -20°C. Prevent repeated freeze thaw cycles.
- **Inhibitor NNGH (2 mM):** Aliquot and store at -20°C.

VI. MMP-9 Inhibitor Screening Protocol:

1. **MMP-9 enzyme Control (EC):** Dilute the reconstituted MMP-9 to 1:5 with the MMP-9 Assay Buffer. Prepare the enzyme Control (EC) by mixing 5 µl of the diluted MMP-9 with 45 µl of MMP-9 Assay Buffer.
2. **Background Control (BC):** Prepare a Background Control (BC) with 50 µl of MMP-9 Assay Buffer alone.
3. **Screening Compound and Inhibitor Control (IC) preparation:** Dissolve candidate inhibitors into an appropriate solvent to make a 100X stock solution. Prepare Inhibitor Samples (S) by mixing 1 µl candidate Inhibitor stock with 5 µl diluted MMP-9 and 44 µl MMP-9 Assay Buffer. Prepare an Inhibitor Control (IC) by mixing 2 µl NNGH with 5 µl diluted MMP-9 and 43 µl MMP-9 Assay Buffer. **Note:** Solvents used to solubilize the inhibitors might affect the enzymatic activity. If solvent effect on the enzymatic activity is a concern, prepare Solvent Control well(s) (SC) with the same final concentration of the solvent(s) as in the inhibitor sample(s) containing 5 µl of the diluted MMP-9 as in the enzyme control. Make up the volume to 50 µl with the assay buffer.
4. **Reaction Mix Preparation:** Prepare 50 µl Reaction Mix for each well to be analyzed :

MMP-9 Assay Buffer	49 µl
MMP-9 Substrate	1 µl

   Add 50 µl of the Reaction Mix into all the wells. Mix well.
5. **Measurement:** Measure the fluorescent signal Ex/Em = 325/393 nm in a kinetic mode at 37°C for 30-60 min.
6. **Calculation:** Choose two time points T_1 & T_2, (at least 10 min. apart) in the linear range of enzyme kinetics and obtain the corresponding ΔRFU (RFU2 –RFU1) during the reaction time ΔT (T2 - T1) for Enzyme Control ($\Delta RFU_{(EC)}$), and Test Inhibitor ($\Delta RFU_{(Test\ Inhibitor)}$). Use these values to obtain the percentage of inhibition.

$$\textbf{\% Relative Inhibition} = \frac{\Delta RFU_{(EC)} - \Delta RFU_{(Test\ Inhibitor)}}{\Delta RFU_{(EC)}} \textbf{ X 100}$$

Notes:

- Irreversible inhibitors that inhibit the MMP-9 activity completely at the tested concentration will have ΔRFU = 0 and thus the % Inhibition will be 100%.
- Subtract Background Control (BC) reading from the Enzyme Control (EC) and Inhibitor (S). If the data obtained from the solvent control(s) is significantly different from the EC, use this data instead of the EC data in the equation above.
- If Solvent control (SC) values are significantly different from the EC, use these values in the equation above after subtracting BC.

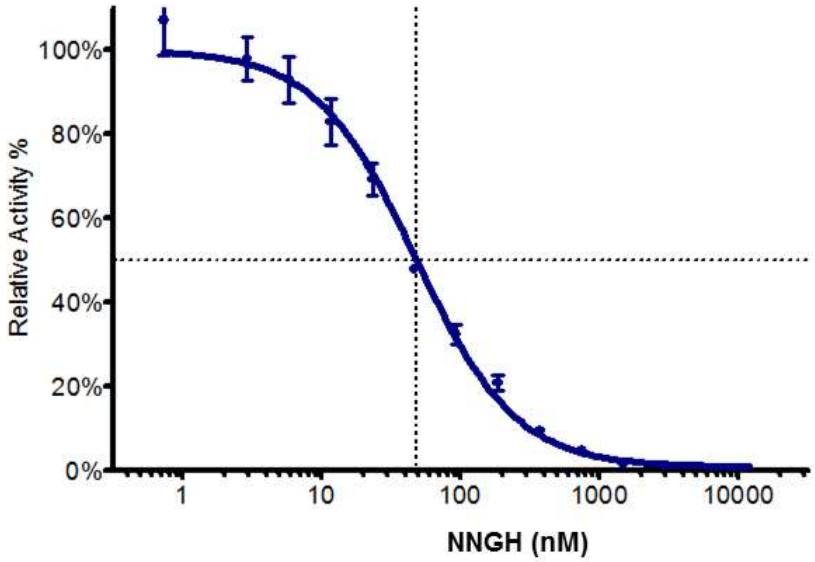

Figure: Inhibition of MMP-9 activity by NNGH (IC_{50} = 47.8 nM), a MMP inhibitor (Cat # 2569). Assay performed following kit protocol.

VII. RELATED PRODUCTS:

Active MMP-9, human recombinant (7867)
MMP-9, Human CellExp™, human recombinant (7246)
Collagenase Inhibitor Screening Kit (K833)
Collagen-I, human recombinant (4796)
Collagen-III, human recombinant (4797)
MMP-1 Antibody (5781)
MMP-9 Antibody (3529, 3969, 5565)
MMP-17 Antibody (3537)
MMP-9 inhibitor (1981)
MMP-9, Human Recombinant (7789)
Self-Quenched BODIPY FL Conjugate of BSA (Green) (7932)
Self-Quenched BODIPY Gelatin (7935-30)
MMP-1 Inhibitor Screening Kit (K794)
MMP-8 (rat) ELISA Kit (K4743)
MMP-2 Antibody (5562)
MMP-11 Antibody (3531R)
MMP-III Inhibitor, NNGH (2569)

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