

ab284517 – MMP-9 Inhibitor Screening Kit (Fluorometric)

For the screening of potential MMP-9 inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab284517>

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
MMP-9 Assay Buffer	25 mL	-20°C
MMP-9 Substrate (4 mM)	100 µL	-20°C
MMP-9 (lyophilized)	1 vial	-20°C
Inhibitor (NNGH 2 mM)	50 µL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

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 dH₂O). 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.

Assay Protocol

MMP-9 enzyme Control (EC):

1. Dilute the reconstituted MMP-9 to 1:5 with the MMP-9 Assay Buffer.
2. Prepare the enzyme Control (EC) by mixing 5 µL of the diluted MMP-9 with 45 µL of MMP-9 Assay Buffer.

Background Control (BC):

1. Prepare a Background Control (BC) with 50 µL of MMP-9 Assay Buffer alone.

Screening Compound and Inhibitor Control (IC) preparation:

1. Dissolve candidate inhibitors into an appropriate solvent to make a 100X stock solution.
2. Prepare Inhibitor Samples (S) by mixing 1 µL candidate Inhibitor stock with 5 µL diluted MMP-9 and 44 µL MMP-9 Assay Buffer.
3. Prepare an Inhibitor Control (IC) by mixing 2 µL NNGH with 5 µL diluted MMP-9 and 43 µL MMP-9 Assay Buffer.

Δ 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.

Reaction Mix:

1. Prepare 50 µL Reaction Mix for each well to be analyzed :

	Reaction Mix
MMP-9 Assay Buffer	49 µL
MMP-9 Substrate	1 µL

2. Add 50 µL of the Reaction Mix into all the wells. Mix well.

Measurement

Measure the fluorescent signal Ex/Em = 325/393 nm in a kinetic mode at 37°C for 30-60 min.

Calculation:

1. Choose two time points T₁ & T₂, (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 (ΔRFU_(EC)), and Test Inhibitor (ΔRFU_(Test Inhibitor)).
2. Use these values to obtain the percentage of inhibition.

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

Δ Notes:

- a) Irreversible inhibitors that inhibit the MMP-9 activity completely at the tested concentration will have ΔRFU = 0 and thus the % Inhibition will be 100%.
- b) 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.
- c) If Solvent control (SC) values are significantly different from the EC, use these values in the equation above after subtracting BC.

Technical Support

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