ab283367 – MMP2 Inhibitor Screening Kit (Fluorometric)

For the screening of potential MMP2 inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283367

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 1 year.

Materials Supplied

Item	Quantity	Storage Condition
MMP-2 Assay Buffer	25 mL	-20°C
MMP-2 Substrate	100 µl	-20°C
Recombinant MMP-2	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:

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

Reagent Preparation

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

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 dH2O). 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

Assay Protocol

- MMP-2 is temperature sensitive. Set up assay(s) and plate on ice prior to measurement.

Pre-activate Recombinant MMP-2:

1. 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.
2. Add 50 µl of the diluted MMP-2 enzyme into desired wells of a 96-well white plate labelled as Sample, Solvent Control, Inhibitor Control and Enzyme Control respectively.
3. Incubate at 37°C for 30 min to activate the enzyme

Screening Test Inhibitor(s):

1. 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.
2. 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.

Δ ***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).*

Enzyme, Background and Inhibitor Control Preparation:

1. 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.
2. **IC50 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.

Test Compound preparation:

1. Dissolve the test compound in appropriate solvent.
2. Prepare at such concentration so volume of test compound solution added to a well is no more than 5 µl in the final 100 µl reaction volume per well.
3. Add 2-5 µl test compound to each well of the 96-well white microplate. For "Solvent Control", add 2-5 µl of the solvent used to prepare test compound solution at its final concentration in test wells, and for "Inhibitor Control" add 2 µl of Trichostatin A, the provided HDAC5 inhibitor.
4. Bring up the volume to 50 µl in each well by adding distilled water. For the "Enzyme Control" add 50 µl distilled water to a well. Transfer the plate onto ice.

Δ **Note**: *DMSO Concentration, up to 5% Final Concentration, does not adversely inhibit the activity of HDAC5.*

Reaction Mix:

1. Mix enough reagents for the number of assays to be performed, immediately before adding to the plate.
2. Prepare just enough for the number of reactions being run immediately.
3. Keep the vial containing the reaction mix on ice while adding it to the well of the 96 well plate. For each well, prepare 25 µl Mix containing:

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

4. Mix well and add 25 µl of the Reaction Mix to wells containing the Sample, Solvent control, Inhibitor control, Enzyme Control and background control wells. The total reaction volume is 100 µl/well.

Measurement

Start recording fluorescence in a kinetic mode at Ex/Em= 325/393 nm after adding the substrate at 1 min intervals for 30-60 minutes at 37°C.

Calculation:

1. Obtain ΔRFU for all Test Inhibitors, Enzyme Control, Solvent Control and Inhibitor Control by subtracting RFU at time t1 from RFU at time t2, such that t2 and t1 is within a linear range of the assay.
2. Calculate the slope for all Samples including Enzyme Control [EC] by dividing ΔRFU by time Δt (t2 – t1). If Solvent Control [SC] slope is significantly different from Enzyme Control [EC] slope, use its values instead of EC in the calculations shown below.

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

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

Technical Support

For all technical or commercial enquiries please go to:

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