ab308243 – MMP-13 Inhibitor Screening Kit (Fluorometric)

Screening or characterizing MMP-13 inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: abcam website

Storage and Stability

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

Materials Supplied

Item	Quantity	Storage Condition
MMP-13 Assay Buffer	25 ml	RT
MMP-13 Substrate (Avoid light)	100 µl	-20°C
Recombinant MMP-13	25 µl	-20°C
MMP-13 Inhibitor (2 mM) (Avoid light)	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:

- DMSO
- 96-well white plate with flat bottom (low/medium binding)
- Multi-well spectrophotometer (Fluorescent plate reader)

Reagent Preparation

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.

MMP-13 Inhibitor Screening Protocol:

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 labelled as Sample, Solvent Control, Inhibitor Control and Enzyme Control respectively.

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.

Δ 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).*

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.

IC_{50} 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.

MMP-13 Substrate Mix Preparation:

Mix enough Substrate Mix for the number of assays to be performed. Prepare 50 µl Substrate Mix per reaction.

	Substrate Mix
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.

Measurement:

Measure fluorescence in kinetic mode at Ex/Em = 325/393 nm at 1 min intervals for 30-60 min at 37 °C.

Calculation:

Obtain ΔRFU for all Test Samples [S], Enzyme Control [EC], Solvent Control [SC] and Inhibitor Control [IC] by subtracting RFU at time t_1 from RFU at time t_2, such that t_2 and t_1 is within a linear range of the assay. Calculate the slope for all Samples including Enzyme Control [EC] by dividing ΔRFU by time Δt (t_2 - t_1). If [SC] slope is significantly different from [EC] slope, use its values instead of EC in the calculations shown below.

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

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

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)