

ab118973

**MMP-1 Inhibitor
Screening Kit**

Instructions for Use

For the rapid, sensitive and accurate detection of MMP-1 inhibition by various compounds.

This product is for research use only and is not intended for diagnostic use.

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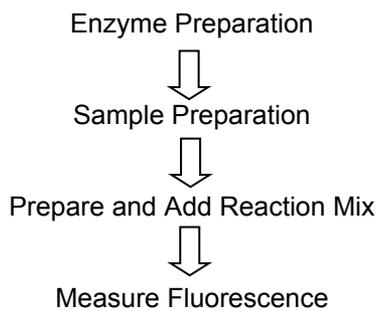
1. Overview

The Matrix metalloproteinase-1 (MMP-1, Interstitial collagenase, fibroblast collagenase) is a member of a multigene family of calcium-dependent, zinc-containing endoproteinases, the matrix metalloproteinases (MMPs). The MMPs are responsible for the degradation of the extracellular matrix (ECM) including collagens, elastins, gelatin, matrix glycoproteins and proteoglycan during normal development and disease processes. MMPs are regulated by hormones, growth factors and cytokines. MMP-1 belongs to the subclass, the collagenases, and along with MMP-8, and MMP-13 are the only members of the MMP family that are capable of degrading the types I, II and III interstitial collagens with high efficiency. These collagens are primarily found in bone, cartilage and skin.

In Abcam's MMP-1 Inhibitor Screening Kit, MMP-1 hydrolyzes a specific FRET substrate to release a quenched fluorescent group, which can be detected at Em/Ex = 490/520nm. In presence of potent MMP-1 inhibitors the hydrolyzation of substrate will be inhibited or stopped.

The kit provides a rapid, simple, sensitive, and reliable test suitable as a high throughput screening assay of MMP-1 inhibitors. For comparison of the relative efficacy of test inhibitors, a control inhibitor, GM 6001 ($IC_{50} = 0.4$ nM for MMP-1) is included.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer	25 mL
Substrate	0.2 mL
MMP-1 Enzyme (Lyophilized)	1 vial
Inhibitor Control (1 μ M GM 6001)	100 μ L

* Store kit at -20°C , protect from light.

- Warm the assay buffer to room temperature before use.
- Briefly centrifuge vials before opening.
- Read the entire protocol before performing the assay.

MMP-1 ENZYME: Reconstitute the MMP-1 enzyme with 220 μ l Assay Buffer. Aliquot and store the MMP-1 stock solution at -80°C . Avoid repeated freeze/thaw cycles. Use within one week.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader
- 96-well plate
- Orbital shaker

4. Assay Protocol

1. Enzyme Preparation:

For each well, prepare a total 50 μ l MMP-1 enzyme solution comprised of:

Assay Buffer	48 μ l
MMP-1 enzyme stock solution	2 μ l

2. Sample Preparation:

Dissolve candidate compounds into a proper solvent. Dilute to 4X the final desired test concentration with Assay Buffer.

For Inhibitor Control, dilute Inhibitor Control Stock 1:25 with Assay Buffer.

Add 25 μ l diluted test compounds, Inhibitor Control or Assay Buffer into MMP-1 enzyme wells as sample screen, Inhibitor Control, or Enzyme Control. Mix well and incubate for 5 min at 37°C.

3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare a total 25 μ l Reaction Mix:

Assay Buffer	23 μ l
Substrate	2 μ l

Add 25 μ l of the Reaction Mix into each reaction well, mix, measure immediately.

4. Measurement: Read Ex/Em = 490/520 nm R_1 at T_1 . Read R_2 again at T_2 after incubating the reaction at 37°C for 30 min, protected from light.

The RFU of fluorescence generated by hydrolyzation of substrate is:

$$\Delta\text{RFU} = R_2 - R_1$$

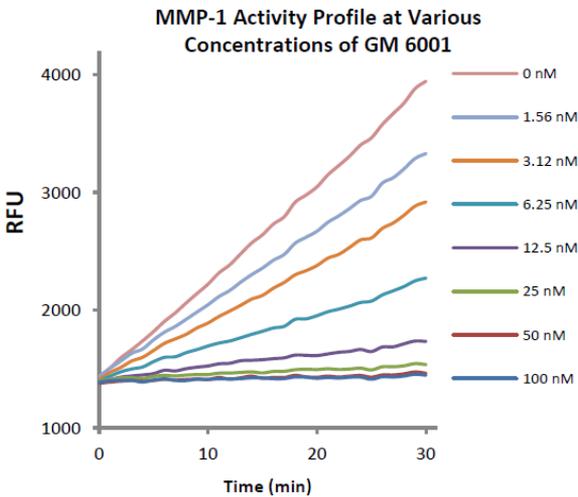
Note:

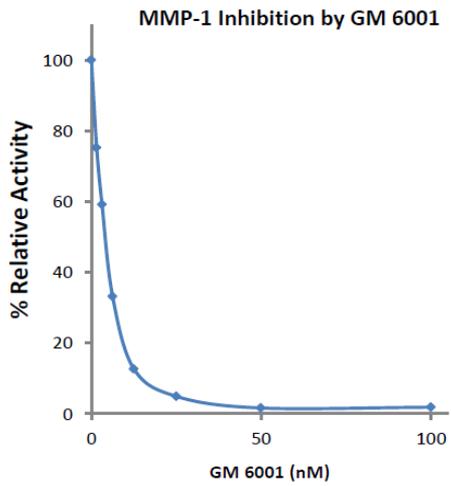
It is recommended to read kinetically to choose the R_1 and R_2 within the linear range.

5. Data Analysis

Set the Δ RFU of Enzyme Control as the 100 % Relative Activity Value and calculate the relative activity for each candidate inhibitor as follows:

$$\% \text{ Relative Activity} = \frac{\Delta\text{RFU of Candidate}}{\Delta\text{RFU of Enzyme Control}} \times 100\%$$





GM6001 Inhibits MMP-1 Activity at Different Concentrations

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms) Luminescence: White plates Colorimetry: Clear plates If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the 10kDa spin column (ab93349)
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select “*contact us*” on www.abcam.com for the phone number for your region).

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