

MMP-1 Inhibitor Screening Kit (Fluorometric) (#BN01010)

(Catalog #BN01010; 100 assays; Store kit at -20 °C)

I. Introduction:

The Matrix metalloproteinase-1 (MMP-1, Interstitial collagenase, fibroblast collagenase) is a member of a multigene family of calcium-dependent, zinc-containing endoproteases, 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 Assay Genie's MMP-1 Inhibitor Screening Kit, MMP-1 hydrolyzes a specific FRET substrate to release a quenched fluorescent group, which can be detected at Ex/Em = 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 (IC₅₀ = 0.4 nM for MMP-1) is included.

II. Kit Contents:

Components	100 assays	Cap Color	Part Number
MMP1 Assay Buffer	25 ml	WM	BN01010-1
MMP1 Substrate	0.2 ml	Red	BN01010-2
MMP-1 Enzyme	1 vial	Green	BN01010-3
Inhibitor Control (1 µM GM 6001)	100 µl	Purple	BN01010-4

III. Storage and Handling:

Store the kit at -20°C, protected from light. Allow the Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol before performing the assay.

IV. Reagent Preparation:

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

V. MMP-1 Inhibitor Screen Assay Protocol:

1. Enzyme Preparation:

For each well, prepare a total 50 µl MMP-3 enzyme solution comprised of:
48 µl Assay Buffer
2 µl MMP-1 enzyme stock solution

2. Screen compounds, Inhibitor Control and Enzyme Control preparations:

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 µl 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.

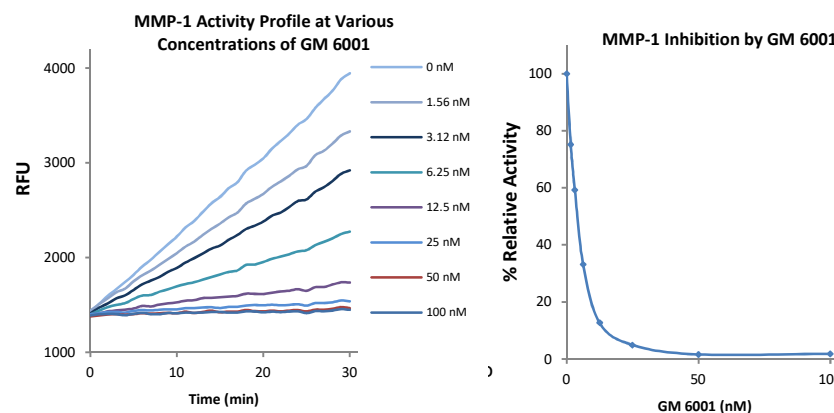
3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare a total 25 µl Reaction Mix:

23 µl Assay Buffer
2 µl Substrate

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

4. **Measurement:** Read Ex/Em = 490/520 nm R₁ at T₁. Read R₂ again at T₂ after incubating the reaction at 37°C for 30 min, protected from light. The RFU of fluorescence generated by hydrolyzation of substrate is $\Delta\text{RFU} = R_2 - R_1$. It is recommended to read kinetically to choose the R₁ and R₂ within the linear range. Set the ΔRFU of Enzyme Control as the 100 % Relative Activity Value and calculate the relative activity for each candidate inhibitor as follows:

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



FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples 	<ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed • Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots 	<ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		