MMP-3 Inhibitor Screening Kit (Fluorometric)

(Catalog #K793-100; 100 assays; Store kit at -20°C)

I. Introduction:

The matrix metalloproteinase-3 (MMP-3, stromelysin-1) exhibits a number of activities that would make it a particularly good tumor promoter. Like several other MMPs, MMP-3 was first cloned and later recloned as a cancer-specific gene. In addition to degrading numerous extracellular matrix components, MMP-3 can activate gelatinase B, the collagenases and several serpin-type serine proteinase inhibitors. Moreover, it can release a number of cell surface molecules, including E-cadherin, a known contributor to cancer development. In BioVision's MMP-3 Inhibitor Screening Assay Kit, MMP-3 hydrolyzes a specific FRET substrate to release the quenched fluorescent group Mca, which can be detected fluorometrically at Ex/Em = 325/393 nm. The kit provides a rapid, simple, sensitive, and reliable test suitable as a high throughput screening assay of MMP-3 inhibition. In addition, we also offer a human recombinant MMP-3 enzyme (Biovision #7783) and a MMP-3 Activity Assay Kit (Biovision #K783-100), separately.

II. Kit Contents:

Components	100 assays	Cap Code	Part Number
MMP-3 Assay Buffer	25 ml	WM	K793-100-1
MMP-3 Substrate	200 µl	Red	K793-100-2
MMP-3 Enzyme (lyophilized)	1 vial	Green	K793-100-3
Inhibitor Control (0.1 mM GM6001)	20 µl	Purple	K793-100-4

III. Storage and Handling:

Store the kit at -20°C, protect from light. Allow 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-3 Enzyme: Reconstitute the MMP-3 enzyme into 220 µl assay buffer. Aliquot and store the MMP-3 stock solution at -80°C. Avoid repeated freeze/thaw cycles. Use within one week.

V. MMP-3 Inhibitor Screening Assay Protocol:

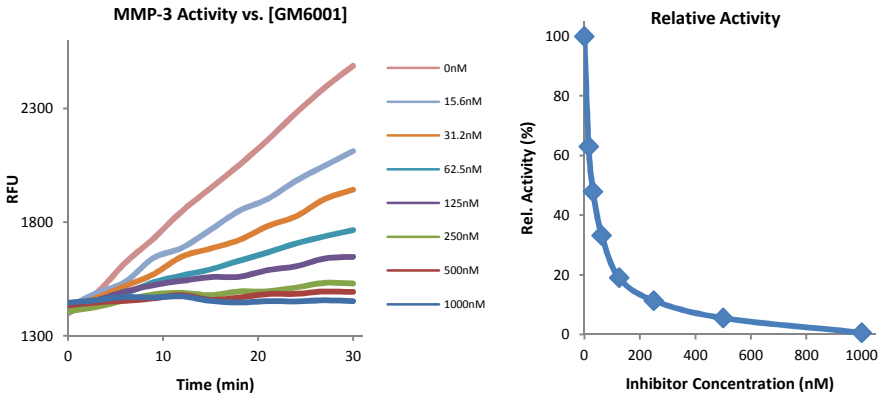
1. **Inhibitor Compounds, Inhibitor Control and Blank Control Preparations:** Dissolve candidate compounds into proper solvent. Dilute to 2X concentration with Assay Buffer. Add 50 µl diluted compounds solution into MMP-3 enzyme wells. For Inhibitor Control, use 2 µl and dilute to 50 µl with Assay Buffer. Use Assay Buffer alone for Blank Control. Mix well.
2. **MMP-3 Enzyme Solution:** Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl MMP-3 enzyme solution.

   48 µl Assay Buffer
   2 µl MMP-3 MMP-3 Stock Solution

   Add 50 µl of the MMP-3 enzyme solution to each well. Incubate candidate compounds-enzyme mixes, inhibitor control-enzyme mix and blank control for 10 min at 37°C.
3. **Substrate:** Dilute Substrate 1:5 with Assay Buffer. Add 10 µl diluted substrate into each well. Mix well.
4. **Measurement:** Read Ex/Em = 325/393 nm R_1 at T_1. Read R_2 again at T_2 after incubating the reaction at 37°C for 30 min, protect from light. The RFU of fluorescence generated by hydrolyzation of substrate is $\Delta RFU = R_2 - R_1$.
5. **Calculation:** Set the ΔRFU of blank control as the 100 %, and calculate the relative activity remaining with candidate compounds as follows.

$$\textbf{Activity Remaining} = \frac{\Delta RFU \text{ of candidate}}{\Delta RFU \text{ of blank}} \times 100\ \%$$

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



RELATED PRODUCTS:

- Human recombinant proteins: MMP-1, -2, -3, -8, -9, -11, -12, -13
- MMP antibodies to: MMP-1, -2, -3, -8, -9, -11, -12, -13, -17, -19
- MMP blocking peptides to: MMP-3, -8, -9, -11, -12
- MMP-3 Inhibitor GM6001
- MMP-3 Activity Assay Kit

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

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GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• 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.		