

# MMP-3 Inhibitor Screening Kit

(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 re-cloned 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	K793-100-2
Inhibitor Control (0.1 mM GM6001)	20 µl	Purple	K793-100-2

## 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:

- 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.
- 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

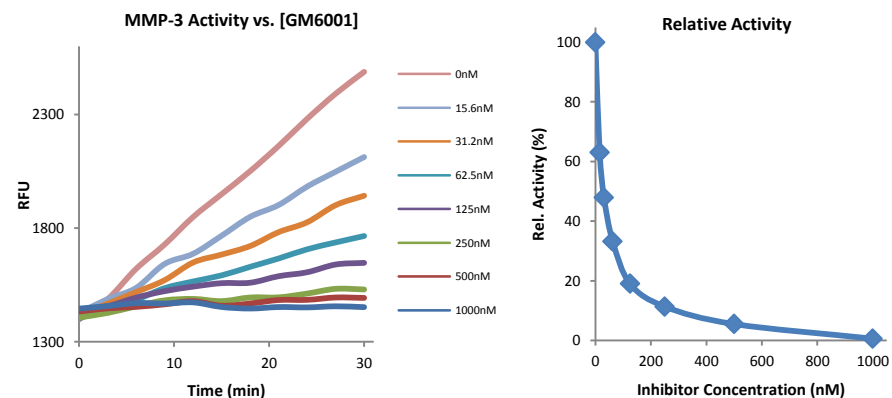
2 µl MMP-3 Stock Solution

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.

- Substrate:** Dilute Substrate 1:5 with Assay Buffer. Add 10 µl diluted substrate into each well. Mix well.
- Measurement:** Read Ex/Em = 325/393 nm R<sub>1</sub> at T<sub>1</sub>. Read R<sub>2</sub> again at T<sub>2</sub> after incubating the reaction at 37°C for 30 min, protect from light. The RFU of fluorescence generated by hydrolyzation of substrate is ΔRFU = R<sub>2</sub> - R<sub>1</sub>.
- Calculation:** Set the ΔRFU of blank control as the 100 %, and calculate the relative activity remaining with candidate compounds as follows.

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

It is recommended to read kinetically to choose the R<sub>1</sub> and R<sub>2</sub> 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.**

**GENERAL TROUBLESHOOTING GUIDE:**

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

**Note:** The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.