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Product Information

MMP-3 Inhibitor Screening Kit (Fluorometric)

Catalog Number **MAK294**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Matrix metalloproteinase-3 (MMP-3 or stromelysin-1) exhibits a number of activities that would make it a particularly good tumor promoter. Similar to 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 the 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 (λ_{ex} = 325 nm/λ_{em} = 393 nm). The kit provides a rapid, simple, sensitive, and reliable test suitable as a high throughput screening assay of MMP-3 inhibition.

Components

The kit is sufficient for 100 assays in 96 well plates.

MMP-3 Assay Buffer Catalog Number MAK294A	25 mL
MMP-3 Substrate Catalog Number MAK294B	200 µL
MMP-3 Enzyme Catalog Number MAK294C	1 vial
Inhibitor Control (0.1 mM GM6001) Catalog Number MAK294D	20 µL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates for this assay
- Fluorescence multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Allow Assay Buffer to warm to room temperature before use. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

MMP-3 Enzyme - Reconstitute the MMP-3 enzyme with 220 µL of assay buffer. Aliquot and store the MMP-3 stock solution at –80 °C. Avoid repeated freeze/thaw cycles. Use within one week.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure

All samples and standards should be run in duplicate.

<u>Inhibitor Compounds, Inhibitor Control, and Blank Control Preparations</u>

Dissolve candidate compounds into proper solvent. Dilute to 2× final concentration with Assay Buffer. Add 50 µL of diluted compound solutions into MMP-3 enzyme wells. For Inhibitor Control, use 2 µL and dilute to 50 µL of 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 of MMP-3 enzyme solution.

Substrate
Dilute Substrate 1:5 with Assay Buffer. Add 10 μL of diluted substrate into each well. Mix well.

Measurement
Read fluorescence (RFU_1, λ_{ex} = 325 nm/λ_{em} = 393 nm) at T_1. Read RFU_2 again at T_2 after incubating the reaction at 37 °C for 30 minutes, protected from light. The fluorescence generated by hydrolysis of the substrate is $\Delta RFU = R_2 - R_1$.

Results
Calculation
The ΔRFU of blank control is set as the 100 % activity. 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_1 and R_2 within a linear range.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	It is recommended to use black plates for this assay.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly 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	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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