RayBio® MMP-3 Activity Assay Kit

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RayBio® MMP-3 Activity Assay Kit Protocol

(Cat#: 68AT-MMP3-S100)

RayBiotech, Inc.

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TABLE OF CONTENTS

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 RayBiotech's MMP-3 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 which can also be used as a high throughput assay of MMP-3 activity. The assay sensitivity is < 50 µU. This kit can be used with our MMP-3 inhibitor, GM6001 as a control. In addition,

we also offer a MMP-3 inhibitor Screening Kit (RayBiotech # 68SR-MMP3-S100), separately.

II. REAGENTS

Components	MMP3-S100	Cap Code	Part Number
MMP-3 Assay Buffer	25 ml	WM	Item A
MMP-3 Substrate	200 µl	Red	Item B
MCA standard (1mM)	20 µl	Yellow	Item C
MMP-3 Positive Control (lyophilized)	1 vial	Green	Item D

III. STORAGE & PREPARATION

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 PRPARATION:

MMP-3 Positive Control: Reconstitute with 100 µl assay buffer. Aliquot and store at -20^{o}C. Avoid repeated freeze/thaw cycles. Use within one week.

V. MMP-3 Assay Protocol:

1. **Standard Curve Preparation:**

   Mix 5 µl 1mM MMP Mca Standard with 495 µl MMP-3 Assay Buffer to generate a 10 µM standard solution. Add 0, 10, 20, 30, 40, 50 µl to each well individually. Adjust to a final volume of 100 µl/well with Assay Buffer to generate 0, 0.1, 0.2, 0.3, 0.4, 0.5 nmol/well of Mca Standard. Read fluorometrically at Ex/Em=325/393 nm.

2. **Sample Preparations:**

   Tissues (50 mg) or cells (1×10^6) can be homogenized in ~ 200 µl ice-cold MMP-3 Assay Buffer then centrifuged to remove insoluble material at 13,000 *g*, 10 minutes. Serum sample can be directly diluted in the MMP-3 Assay Buffer. Prepare test samples of up to 50 µl/well with MMP-3 Assay Buffer in a 96-well plate. We suggest testing several doses of your sample to make sure the readings are within the Standard Curve range. For Positive Control use 5-10 µland adjust well volume to 50 µl with Assay Buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix:

   48 µl MMP-3 Assay Buffer

   2 µl MMP-3 substrate

   Add 50 µl of the Reaction Mix to each well containing the samples and positive controls. 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 room temperature for 60 min (or incubate longer time if the sample activity is low), protect from light. The RFU of fluorescence generated by hydrolyzes of the substrate is $\Delta RFU = R_2 - R_1$. It is recommended to read kinetically to choose the R_1 and R_2 values that fall within the linear range of the Standard Curve.

5. **Calculation:** Subtract the 0 Standard from the Standard readings. Plot the Standard Curve and apply the ΔRFU to the standard curve to get B nmol of Mca (amount of unquenched Mca generated between T_1 and T_2).

MMP-3 Activity = $\frac{B}{(T2-T1)\times V}$ **×Sample Dilution Factor = nmol/min/ml = mU/ml**

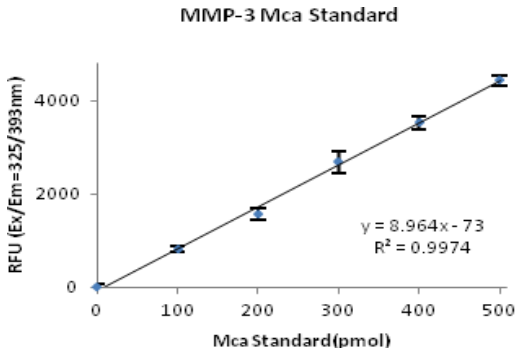
Where: **B** is the Mca amount from MMP Mca Standard Curve (in nmol).

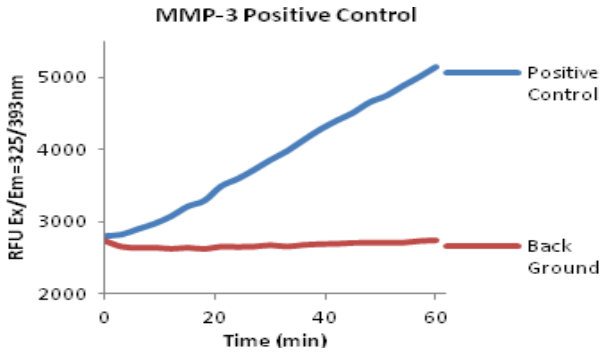
T_1 is the time of the first reading (R_1) (in min).

T_2 is the time of the second reading (R_2) (in min).

V is the pretreated sample volume added into the reaction well (in ml).

Unit Definition: One unit is defined as the amount of enzyme that will generate 1.0 µmol of unquenched Mca per minute at room temperature.


MMP-3 Mca Standard
RFU (Ex/Em=325/393nm)
4000
2000
0
0
100
200
300
400
500
Mca Standard(pmol)
y = 8.964x - 73
R² = 0.9974


MMP-3 Positive Control
RFU Ex/Em=325/393nm
5000
4000
3000
2000
0
20
40
60
Time (min)
Positive Control
Back Ground

VI. GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• 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	• 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	• 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	• 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	• 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	• 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	• 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	• 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	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• 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.		

NOTES:

This product is for research use only.