

Description

The MMP7 (Matrix MetalloProteinase 7) Fluorogenic Assay Kit is designed to measure MMP7 protease activity for screening and profiling applications, in a homogeneous assay with no time-consuming washing steps. The kit contains enough recombinant MMP7, substrate and assay buffer for 100 reactions.

Background

MMP7 (also known as matrix metalloproteinase 7 or pump-1 protease) is a member of the large family of matrix metalloproteinases (MMP) responsible for the breakdown of extracellular matrix proteins such as casein, collagen, and fibronectin. Most MMPs are secreted as inactive pro-proteins and are activated when cleaved by other extracellular proteinases. MMP7 cleaves fibronectin, gelatin and elastin, but not collagen. MMPs are involved in tissue remodeling and in the escape of metastatic cells from the primary tumor and angiogenesis. MMP7 can activate MMP2 and MMP9, and upregulation of MMP7 is found in many carcinomas. They are candidate therapeutic targets in various diseases related to tissue remodeling including cancer and inflammatory diseases.

Applications

Screen or profile small molecule inhibitors of MMP7.

Supplied Materials

Catalog #	Name	Amount	Storage
	Recombinant MMP7*	7.5 µg	-80°C
79919	1 mM MMP Substrate	10 µl	-80°C
79917	MMP Assay Buffer 1	25 ml	-20°C
79685	Black 96-well plate	1	Room Temperature

*The concentration of the protein is lot-specific and will be indicated on the tube.

Materials Required but Not Supplied

- Fluorescent microplate reader capable of reading $\lambda_{exc}/\lambda_{em}=328\text{ nm}/393\text{ nm}$
- Adjustable micropipettor and sterile tips
- 30°C incubator

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

The final concentration of DMSO in the assay should not exceed 1%.

Assay Protocol

- All samples and controls should be tested in duplicate.
- If the assay plate is going to be used more than once, prepare enough reagents for this portion of the assay and aliquot the remaining undiluted reagents into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C or at -20°C as appropriate.

1. Thaw **MMP Assay Buffer 1** and **1 mM MMP Substrate**.
2. Dilute **1 mM MMP Substrate** 100-fold in **MMP Assay Buffer 1**, to make a 10 µM solution.
3. Prepare the **Substrate solution** (25 µl/well): N wells x (20 µl of **MMP Assay Buffer 1** + 5 µl of diluted (**10 µM**) **MMP Substrate**). Final concentration of the **10 µM MMP Substrate** in a 50 µl reaction is 1 µM.
4. Add 25 µl of Substrate solution to every well.
5. Prepare the Test Inhibitor (5 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

5.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in the MMP Assay Buffer 1, 10-fold more concentrated than the desired final concentrations. MMP Assay Buffer 1 is the Diluent Solution.

OR

5.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor in 100% DMSO at 100-fold the highest desired concentration, then dilute the inhibitor 10-fold in MMP Assay Buffer 1 to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in MMP Assay Buffer 1 to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in MMP Assay Buffer 1 (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

6. Add 5 µl inhibitor solution to each well designated “Test Inhibitor.”
7. Add 5 µl of Diluent Solution to the “Blank” and “Positive Control” wells.
8. Thaw **MMP7** on ice. Briefly spin tube containing enzyme to recover the full content of the tube.

*Note: The concentration is lot-specific and is indicated on the tube. Verify the initial concentration and dilute accordingly. **MMP7** is particularly sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use the diluted protein.*

9. Dilute **MMP7** in **MMP Assay Buffer 1** to 3.2 ng/µl (20 µl/ well).
10. Add 20 µl diluted **MMP7** solution to wells designated as “Positive Control” and “Test Sample.”

11. Add 20 μ l **MMP Assay Buffer 1** to the “Blank” wells.

Component	Blank	Positive Control	Test Inhibitor
Substrate Solution	25 μ l	25 μ l	25 μ l
Test Inhibitor	-	-	5 μ l
1x MMP Assay Buffer 1	20 μ l	-	-
Diluent Solution	5 μ l	5 μ l	-
Diluted MMP7 (3.2 ng/ μ l)	-	20 μ l	20 μ l
Total	50 μl	50 μl	50 μl

12. Incubate at Room Temperature for 30 minutes.

13. Measure the fluorescence intensity in a fluorescence plate reader capable of excitation at a wavelength 328 nm and detection of emission at a wavelength 393 nm. The fluorescence intensity can also be measured kinetically.

14. “Blank” value should be subtracted from all other values.

Example Results

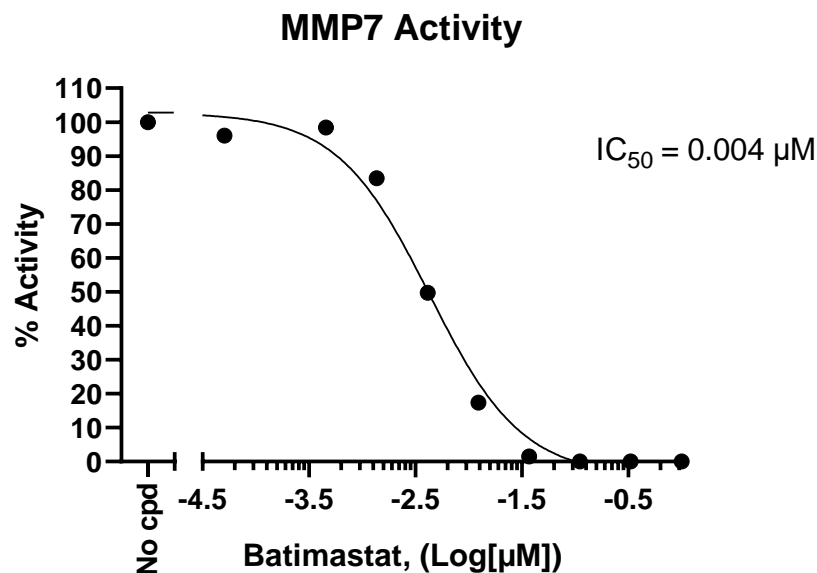


Figure 1: Inhibition of MMP7 Activity by Batimastat (Selleckchem #S7155).

The inhibition of MMP7 activity was measured in the presence of increasing concentrations of inhibitor. The “Blank” value was subtracted from all other values. Results are expressed as the percent of control (activity in the absence of inhibitor, set at 100%).

For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For further questions, please email support@bpsbioscience.com

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
MMP8 Fluorogenic Assay Kit	79929	96 reactions
MMP8, His-Tag, Recombinant	100552	20 µg
MMP26 Fluorogenic Assay Kit	78830	96 reactions