Description

The MMP26 (Matrix metalloproteinase 26) Fluorogenic Assay Kit is designed to measure MMP26 protease activity for screening and profiling applications, in a homogeneous assay with no time-consuming washing steps. The kit contains enough recombinant MMP26 enzyme, substrate, and assay buffer for 96 reactions.

Background

MMP26 (also known as matrilysin-2 or endometase) is a member of the large family of matrix metalloproteinases (MMP) responsible for the breakdown of extracellular matrix proteins such as collagen. Most MMPs are secreted as inactive pro-proteins and are activated when cleaved by extracellular proteinases. MMP26 cleaves collagen type IV, vitronectin, fibronectin, and fibrinogen, among others. It also cleaves MMP9 for activation. MMPs are involved in tissue remodeling and in the escape of metastatic cells from the primary tumor. They are candidate therapeutic targets in various diseases related to tissue remodeling including cancer.

Applications

Screen or profile small molecule inhibitors of MMP26

Supplied Materials

Catalog #	Name	Amount	Storage
	Recombinant MMP26*	1 µg	-80°C
79919	1 mM MMP Substrate	10 µl	-80°C
79917	1x 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 λexc/λem=328 nm/393 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.
- Briefly spin the tubes containing reagents to recover their full contents.
- Note: MMP26 is very sensitive to freeze/thaw cycles. Do not re-use the thawed protein and do not re-use the diluted protein.

1. Thaw **1x MMP Assay Buffer 1** and **1 mM MMP Substrate.**

2. Dilute **1 mM MMP Substrate** 100 fold in **1x MMP Assay Buffer 1**, to make a 10 µM solution.

3. Prepare the **Substrate solution** (25 µl/well): N wells x (20 µl of **1x MMP Assay Buffer 1** + 5 µl of diluted **MMP Substrate at 10 µM**). The Final concentration of **MMP Substrate** in a 50 µl reaction is 1 µM.

4. Add 25 µl of the substrate solution to each 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.

   i) If the Test Inhibitor is water-soluble, prepare serial dilutions in the 1x MMP Assay Buffer, 10-fold more concentrated than the desired final concentrations. For the positive and negative controls, use 1x MMP Assay Buffer (Diluent Solution).

   OR

   ii) 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 1x MMP Assay Buffer 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 1x MMP Assay Buffer to keep the concentration of DMSO constant.
   For positive and negative controls, prepare 10% DMSO in 1x MMP Assay Buffer (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.” Add 5 µl of 10% DMSO in 1x MMP Assay Buffer (Diluent Solution) to “Blank” and “Positive Control” wells.

7. Thaw **MMP26** on ice. Briefly spin the tube containing the enzyme to recover the full content of the tube.

   Notes: The concentration is lot-specific and is indicated on the tube. Verify the initial concentration and dilute accordingly. ***MMP26*** *is particularly sensitive to freeze/thaw cycles. Do not re-use the thawed protein and do not re-use the diluted protein.*

8. Dilute **MMP26** in **1x MMP Assay Buffer 1** at 0.5 ng/µl (10 ng per reaction).

9. Add 20 µl of diluted **MMP26** to wells designated as "Positive Control" and "Test Inhibitor." Add 20 µl of **1x MMP Assay Buffer 1** to the "Blank" wells.

Component	Blank	Positive Control	Test Inhibitor
Substrate Solution	25 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
1x MMP Assay Buffer 1	20 µl	-	-
MMP26 (0.5 ng/µl)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

10. Incubate at room temperature for 30 minutes.

11. 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. "Blank" value is subtracted from all other values.

Example Results

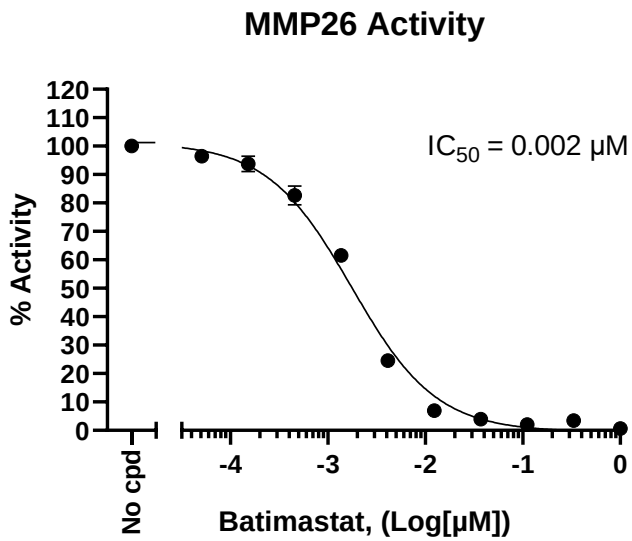

Figure 1: Inhibition of MMP26 Activity by Batimastat.
The inhibition of MMP26 activity was measured in the presence of increasing concentrations of Batimastat *(Selleckchem #S7155)*. The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (activity in the 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

Reference(s)

Ikeda K, *et al*. Proteolytic cleavage of membrane proteins by membrane type-1 MMP regulates cancer malignant progression. *Cancer Sci.* 2023; 114(2): 348-356. PMID: 36336966.

Related Products

Products	*Catalog #*	*Size*
Fluorogenic MMP8 Assay Kit	79929	96 Reactions
3CL Protease, Untagged (SARS-CoV-2) Assay Kit	78042	96 reactions/384 reactions
Activated Protein C (PROC) Assay Kit	78827	96 Reactions
ADAM10 Fluorogenic Assay Kit	78007	96 Reactions
Caspase-3 Homogeneous Assay Kit	80700	96 Reactions