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Data Sheet

Fluorogenic MMP2 Assay Kit

Catalog #79918
Size: 96 reactions

BACKGROUND: MMP2 (matrix metalloproteinase 2), also known as Gelatinase A, is a member of the matrix metalloproteinase (MMP) family involved in the breakdown of type IV collagen, a major structural component of basement membranes. The activity of MMP2 is associated with cancer cell metastasis and other diseases, including Multicentric Osteolysis, Nodulosis, and Arthropathy.

DESCRIPTION: The *Fluorogenic MMP2 Assay Kit* is designed to measure MMP2 activity for screening and profiling applications, in a homogeneous assay with no time-consuming washing steps. The kit comes in a convenient 96-well format, with purified MMP2 enzyme, fluorogenic substrate, and MMP2 assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Component	Amount	Storage	
80213	Recombinant Human MMP2	1 µg	-80°C	***Avoid freeze/ thaw cycles!***
79919	1 mM MMP2 Substrate	10 µl	-80°C	
79920	1X MMP2 Assay Buffer	25 ml	-20°C	
	0.5 M DTT	50 µl	-20°C	
79685	Black, low binding black microtiter plate	1	Room Temperature	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable of reading exc/em=328 nm/393 nm

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

STABILITY: One year from date of receipt when stored as directed.

REFERENCE(S):

1. Björklund, M. and Koivunen, E. 2005 May 25; *Biochimica et Biophysica Acta.* **1755 (1):** 37–69.
2. Olaf, R.F., *et al.* 2004 Dec 17; *Biochimica et Biophysica Acta.* **1705 (2):** 69–89.

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

1) Add 0.5 M DTT to 1X assay buffer so final DTT concentration is 1 mM. For example, add 10 µl of 0.5M DTT to 5 ml 1X assay buffer. (DTT should be added just before use. Prepare only enough DTT-containing buffer as required for the assay. Store the remaining 1X assay buffer at -20°C).

2) Dilute MMP substrate (1 mM) 50-fold in 1 x assay buffer to make a 20 µM solution. Dilute only the amount required for the assay.

3) Prepare the substrate solution: N wells × (20 µl 1X assay buffer (with DTT) + 5 µl diluted (20 µM) MMP2 Substrate).

4) Add 25 µl of the substrate solution to each well (Final concentration of the MMP2 substrate in a 50 µl reaction is 2 µM).

5) Prepare the inhibitor solution.

   The final concentration of DMSO in the assay should not exceed 1%. If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound than the highest concentration you want to test in DMSO. Then make a 10-fold dilution in 1X assay buffer (at this step the compound concentration is 10-fold higher than the final concentration in 10% DMSO). To determine an IC50 or to test lower concentrations of the compound, prepare a series of further dilutions in 1X assay buffer containing 10% DMSO (the final concentration of the DMSO will be 1% in all samples).

   If the inhibitor compound is dissolved in water, make a solution of the compound 10-fold higher than the final concentration in 1X assay buffer (with 1 mM DTT).

Component	Positive Control	Test Sample	Blank
Substrate solution	25 µl	25 µl	25 µl
Test Inhibitor	–	5 µl	–
Inhibitor Buffer (no inhibitor)*	5 µl	–	5 µl
MMP2 (0.2 ng/µl)	20 µl	20 µl	–
1X Assay Buffer	–	–	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

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6) Add 5 µl inhibitor solution to each well designated "Test Sample". Add 5 µl 1X assay buffer or 10% DMSO (depending on which inhibitor solution is used) to "Blank" and "Positive Control" wells.

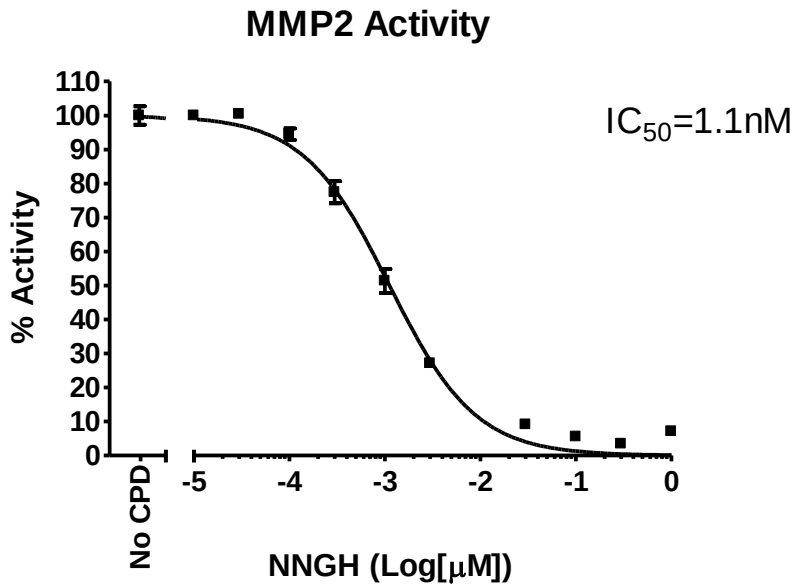
7) Thaw MMP2 on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full content of the tube. Aliquot MMP2 into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. Note: MMP2 enzyme is sensitive to freeze/thaw cycles. Do not re-use diluted enzyme.

8) Dilute MMP2 in 1x assay buffer (with 1 mM DTT) at 0.2 ng/µl (4 ng per reaction).

9) Add 20 µl diluted MMP2 enzyme solution to wells designated as "Positive Control" and "Test Sample". Add 20 µl 1X assay buffer to the "Blank" wells.

10) Incubate at room temperature for 30 minutes. Measure the fluorescence intensity in a microtiter plate-reading fluorimeter 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 OF ASSAY RESULTS:



Inhibition of MMP2 enzyme activity by NNGH, measured using the *Fluorogenic MMP2 Assay Kit (BPS Bioscience #79918).* Fluorescence intensity was measured using a Tecan fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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RELATED PRODUCTS

Product	Cat. #	Size
MMP2, His-Tag (Human)	80213	20 µg
MMP1, His-Tag (Human)	80214	20 µg
MMP3(K45E), His-Tag (Human)	11346	100 µg
MMP8, His-Tag (Human)	100552	100 µg
MMP9(Q279R), His-Tag (Human)	80215	20 µg
MMP3 (K45E) Inhibitor Screening Assay Kit	79907	384 rxns.

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