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Data Sheet

Fluorogenic MMP1 Assay Kit

Catalog #79983
Size: 96 reactions

BACKGROUND: MMP1 (matrix metalloproteinase 1) is a member of the matrix metalloproteinase (MMP) family involved in the degradation of the extracellular matrix. MMP1 is also associated with the regulation of cytokines and chemokines, suggesting a role for MMP1 in inflammation.

DESCRIPTION: The *Fluorogenic MMP1 Assay Kit* is designed to measure MMP1 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 MMP1 enzyme, fluorogenic substrate, and MMP1 assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Component	Amount	Storage	
80214	MMP1, His-tag	4 µg	-80°C	Avoid freeze/ thaw cycles!
79919	1 mM MMP Substrate	10 µl	-80°C	
79917	1X MMP Assay Buffer 1	25 ml	-20°C	
79685	Black, low binding black microtiter plate	1	Room Temperature	

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

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

REFERENCE(S):

- Foley, C.J., and Kuliopulos, A. Mouse Matrix metalloprotease-1a (Mmp1a) Gives New Insight Into MMP Function. *J. Cell Physiol.* 2014 Dec; **229(12)**:1875-80.
- Pardo, A., and Selman, M. MMP-1: The Elder of the Family. *Int. J. Biochem. Cell Biol.* 2005 Feb; **37(2)**:283-8.

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

Fluorescent microplate reader capable of reading exc/em=328 nm/393 nm

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

- 1) Dilute 1 mM MMP substrate 1:100 in 1X assay buffer, to make a 10 μ M solution. Dilute only enough as is required for the assay.
- 2) Prepare the substrate solution: N wells \times (20 μ l 1X assay buffer + 5 μ l diluted (10 μ M) MMP Substrate).
- 3) Add 25 μ l of the substrate solution to each well (Final concentration of the MMP substrate in a 50 μ l reaction is 1 μ M).

Component	Positive Control	Test Sample	Blank
Substrate solution	25 μ l	25 μ l	25 μ l
Test Inhibitor	–	5 μ l	–
10% DMSO in water (Inhibitor buffer)	5 μ l	–	5 μ l
MMP1 (1.6 ng/ μ l)	20 μ l	20 μ l	–
1X Assay Buffer	–	–	20 μ l
Total	50 μl	50 μl	50 μl

- 4) 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.

- 5) Add 5 μ l inhibitor solution to each well designated “Test Sample.” Add 5 μ l of 10% DMSO in water (inhibitor buffer) to “Blank” and “Positive Control” wells.
- 6) Thaw MMP1 on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full content of the tube. Aliquot MMP1 into single use aliquots. Store

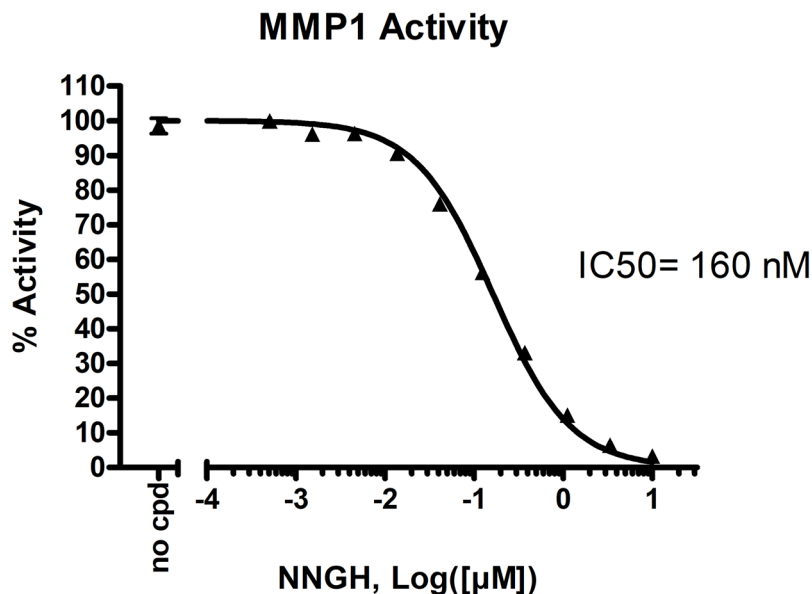
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- remaining undiluted enzyme in aliquots at -80°C . Note: MMP1 enzyme is sensitive to freeze/thaw cycles. Do not re-use diluted enzyme.
- 7) Dilute MMP1 in 1x assay buffer at $1.6\text{ ng}/\mu\text{l}$ (32 ng per reaction).
 - 8) Add $20\ \mu\text{l}$ diluted MMP1 enzyme solution to wells designated as "Positive Control" and "Test Sample." Add $20\ \mu\text{l}$ 1X assay buffer to the "Blank" wells.
 - 9) 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 MMP1 enzyme activity by NNGH, measured using the *Fluorogenic MMP1 Assay Kit* (BPS Bioscience #79983). 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

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
MMP1, His-Tag (Human)	80214	20 µg
MMP2, His-Tag (Human)	80213	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.
Fluorogenic MMP2 Assay Kit	79918	96 rxns.
Fluorogenic MMP9 (Q279R) Assay Kit	79915	96 rxns.

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