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Data Sheet

Fluorogenic MMP10 Assay Kit

Catalog #79986
Size: 96 reactions

BACKGROUND: MMP10 (matrix metalloproteinase 10), also known as Stromelysin-2, is a member of the matrix metalloproteinase (MMP) family involved in the degradation of the extracellular matrix. MMP10 moderates inflammation by controlling macrophage activation and is linked to several lung disorders, including pulmonary fibrosis and cystic fibrosis. MMP10 has also been shown to cleave the mutant huntingtin protein associated with Huntington's disease.

DESCRIPTION: The *Fluorogenic MMP10 Assay Kit* is designed to measure MMP10 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 MMP10 enzyme, fluorogenic substrate, and MMP assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Component	Amount	Storage	
100565	MMP10, His-tag	7 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: At least one year from date of receipt when stored as directed.

REFERENCE(S):

1. Batra, J. *et al.* Matrix Metalloproteinase-10/TIMP-2 Structure and Analyses Define Conserved Core Interactions and Diverse Exosite Interactions in MMP/TIMP Complexes. *PLoS One*. 2013 Sep 20; **8(9):e**75836.

2. McMahan, R.S., *et al*. Stromelysin-2 (MMP10) Moderates Inflammation by Controlling Macrophage Activation. *J. Immunol.* August 1, 2016, **197(3):** 899-909.

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

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 µM solution. Dilute only enough as is required for the assay. Store remaining 1 mM substrate in aliquots at -80°C.

2) Prepare the substrate solution: N wells × (20 µl 1X assay buffer + 5 µl diluted (10 µM) MMP Substrate).

3) Add 25 µl of the substrate solution to each well (Final concentration of the MMP substrate in a 50 µl reaction is 1 µM).

Component	Positive Control	Test Sample	Blank
Substrate solution	25 µl	25 µl	25 µl
Test Inhibitor	–	5 µl	–
Inhibitor buffer (usually 10% DMSO in 1X assay buffer	5 µl	–	5 µl
MMP10 (3.2 ng/µl)	20 µl	20 µl	–
1X Assay Buffer	–	–	20 µl
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 (with 1 mM DTT).

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5) Add 5 µl inhibitor solution to each well designated "Test Sample." Add 5 µl of inhibitor buffer (the same solution as the test sample, but without inhibitor) to "Blank" and "Positive Control" wells.

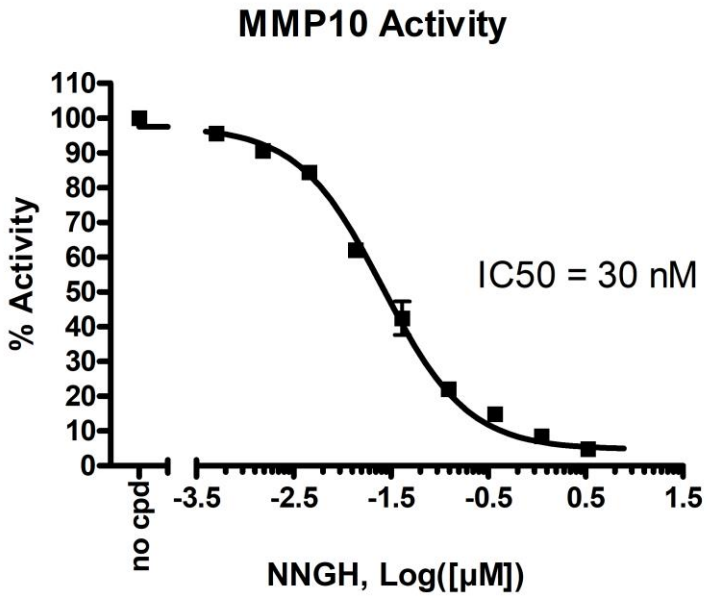
6) Thaw MMP10 on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full content of the tube. Aliquot MMP10 into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. Note: MMP10 enzyme is sensitive to freeze/thaw cycles. Do not re-use diluted enzyme.

7) Dilute MMP10 in 1x assay buffer at 3.2 ng/µl (64 ng per reaction).

8) Add 20 µl diluted MMP10 enzyme solution to wells designated as "Positive Control" and "Test Sample." Add 20 µl 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 MMP10 enzyme activity by NNGH, measured using the *Fluorogenic MMP10 Assay Kit (BPS Bioscience #79986).* 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
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
Fluorogenic MMP3 (K45E) Assay Kit	79907	384 rxns.
Fluorogenic MMP2 Assay Kit	79918	96 rxns.
Fluorogenic MMP8 Assay Kit	79929	96 rxns.
Fluorogenic MMP9 (Q279R) Assay Kit	79915	96 rxns.