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Data Sheet

Fluorogenic MMP11 Assay Kit

Catalog #78020
Size: 96 reactions

BACKGROUND: MMP11 (matrix metalloproteinase 11), also known as Stromelysin-3 (SL-3), is a member of the matrix metalloproteinase (MMP) family involved in the degradation of the extracellular matrix. MMP11 was initially discovered in breast carcinoma and has since been identified in diverse carcinomas and developmental processes. MMP-11 promotes cancer development by inhibiting apoptosis as well as enhancing migration and invasion of cancer cells. MMP11 is activated by furin, an enzyme linked to the entry of the SARS-CoV-2 virus into cells.

DESCRIPTION: The *Fluorogenic MMP11 Assay Kit* is designed to measure MMP11 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 MMP11 enzyme, fluorogenic substrate, and MMP assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Component	Amount	Storage	
	MMP11	20 µg	-80°C	Avoid freeze/ thaw cycles!
79919	MMP Substrate (1 mM)	10 µl	-80°C	
79917	1X MMP Assay Buffer 1	25 ml	-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 $\lambda_{exc}/\lambda_{em}=328\text{ nm}/393\text{ nm}$

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. Peruzzi, Daniela, *et al.* 2009. "MMP11: a novel target antigen for cancer immunotherapy." *Clinical Cancer Research* **15(12)**: 4104-4113.
2. Cheng, Chun-Wen, *et al.* 2010. "The clinical implications of MMP-11 and CK-20 expression in human breast cancer." *Clinica Chimica Acta* **411(3-4)**: 234-241.

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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. Store remaining 1 mM substrate in aliquots at -80°C.
- 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	–
Inhibitor buffer	5 μ l	–	5 μ l
MMP11 (10 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 inhibitor buffer (same solution without inhibitor; usually 10% DMSO in 1X assay buffer) to “Blank” and “Positive Control” wells.
- 6) Thaw MMP11 on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full content of the tube. Aliquot MMP11 into single use aliquots.

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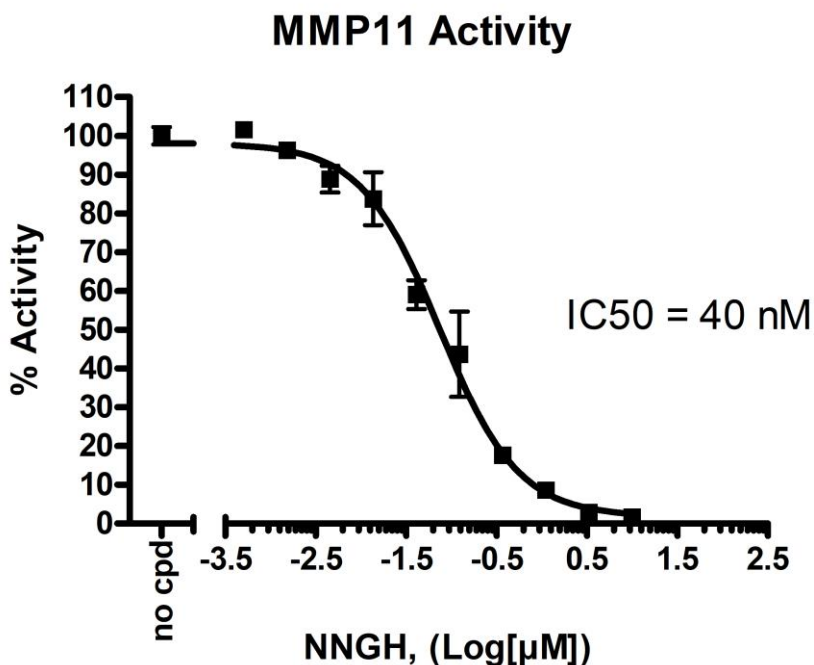
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Store remaining undiluted enzyme in aliquots at -80°C. Note: MMP11 enzyme is sensitive to freeze/thaw cycles. Do not re-use diluted enzyme.

- 7) Dilute MMP11 in 1x assay buffer at 10 ng/μl (200 ng per reaction).
- 8) Add 20 μl diluted MMP11 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 4 hours*. 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.

**Alternatively, you may seal the plate and incubate overnight at room temperature for a greater signal to noise ratio.*

EXAMPLE OF ASSAY RESULTS:



Inhibition of MMP11 enzyme activity by NNGH (Cayman Chemical #16886), measured using the *Fluorogenic MMP11 Assay Kit* (BPS Bioscience #78020). 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
Fluorogenic MMP1 Assay Kit	79983	96 rxns.
Fluorogenic MMP2 Assay Kit	79918	96 rxns.
Fluorogenic MMP3 (K45E) Assay Kit	79907	384 rxns.
Fluorogenic MMP8 Assay Kit	79929	96 rxns.
Fluorogenic MMP9 (Q279R) Assay Kit	79915	96 rxns.
Fluorogenic MMP10 Assay Kit	79986	96 rxns.
Fluorogenic MMP12 Assay Kit	78017	96 rxns.
Fluorogenic MMP13 Assay Kit	79991	96 rxns.
Fluorogenic MMP14 Assay Kit	79993	96 rxns.

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