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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet

Fluorogenic MMP14 Assay Kit

Catalog #79993
Size: 96 reactions

BACKGROUND: MMP14 (matrix metalloproteinase 14) is a member of the matrix metalloproteinase (MMP) family involved in the degradation of the extracellular matrix.MMP14 regulates the activity of multiple extracellular and plasma membrane proteins, influencing cell-cell and cell-extracellular matrix (ECM) degradation and remodeling, cell invasion, and cancer metastasis. MMP14 is highly expressed in most sarcomas, and levels of MMP14 correlate with breast cancer progression. Upregulation of MMP14 also promotes glioma invasion and tumor cell proliferation and plays a role in angiogenesis.

DESCRIPTION: The *Fluorogenic MMP14 Assay Kit* is designed to measure MMP14 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 MMP14 enzyme, fluorogenic substrate, and MMP assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Component	Amount	Storage	
100585	MMP14, His-tag	7 µg	-80°C	***Avoid freeze/ thaw cycles!***
79919	Fluorogenic 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	

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. Chun, Tae-Hwa, *et al.* 2010. "Genetic link between obesity and MMP14-dependent adipogenic collagen turnover." *Diabetes* **59(10):** 2484-2494.
2. Chen, Tzy-Yen, *et al.* 2011. "Role *of* MMP14 gene polymorphisms in susceptibility and pathological development to hepatocellular carcinoma." *Annals of Surgical Oncology* **18(8):** 2348-2356.

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable of reading λexc/λem=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 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 water)	5 µl	–	5 µl
MMP14 (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.

5) Add 5 µl inhibitor solution to each well designated “Test Sample.” Add 5 µl of inhibitor buffer (same solution without inhibitor; usually 10% DMSO in water) to “Blank” and “Positive Control” wells.

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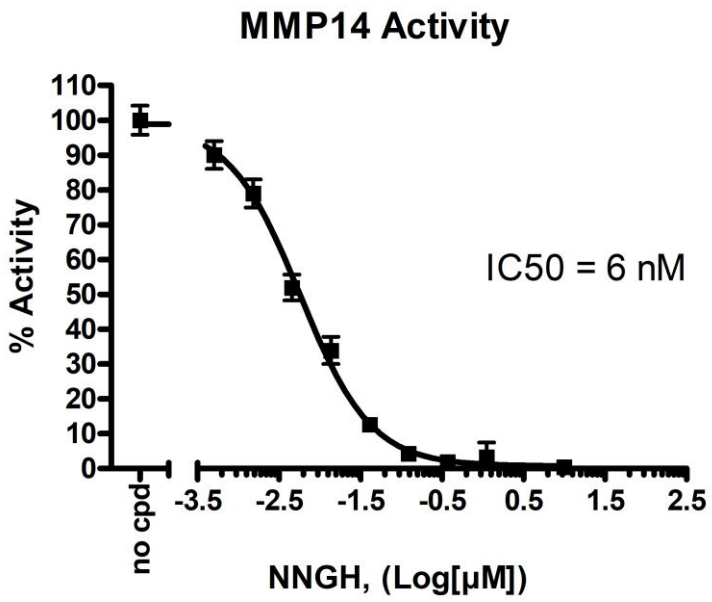
6) Thaw MMP14 on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full content of the tube. Aliquot MMP14 into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. Note: MMP14 enzyme is sensitive to freeze/thaw cycles. Do not re-use diluted enzyme.

7) Dilute MMP14 in 1x assay buffer at 3.2 ng/µl (64 ng per reaction).

8) Add 20 µl diluted MMP14 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 2 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.

EXAMPLE OF ASSAY RESULTS:



Inhibition of MMP14 enzyme activity by NNGH, measured using the *Fluorogenic MMP14 Assay Kit (BPS Bioscience #79993).* 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 MMP1 Assay Kit	79983	96 rxns.
Fluorogenic MMP2 Assay Kit	79918	96 rxns.
Fluorogenic MMP8 Assay Kit	79929	96 rxns.
Fluorogenic MMP9 (Q279R) Assay Kit	79915	96 rxns.
Fluorogenic MMP10 Assay Kit	79986	96 rxns.