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Data Sheet

Fluorogenic MMP13 Assay Kit

Catalog #79991
Size: 96 reactions

BACKGROUND: MMP13 (matrix metalloproteinase 13), also known as collagenase 3, is a member of the matrix metalloproteinase (MMP) family involved in the degradation of the extracellular matrix. MMP13 primarily cleaves type II collagen, and it is expressed in the skeleton where it plays a role in restructuring the collagen matrix for bone mineralization and cartilage turnover. MMP13 is also overexpressed in rheumatoid arthritis, osteoarthritis, and many human carcinomas, suggesting its potential as a therapeutic target.

DESCRIPTION: The *Fluorogenic MMP13 Assay Kit* is designed to measure MMP13 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 MMP13 enzyme, fluorogenic substrate, and MMP assay buffer for 100 enzyme reactions.

COMPONENTS:

| Catalog # | Component | Amount | Storage | |
|-----------|-------------------------------------------|--------|---------------------|-----------------------------------------------|
| 11345 | MMP13, His-tag | 4 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. Wang, M., *et al.* MMP13 is a critical target gene during the progression of osteoarthritis. *Arthritis Res Ther* **15**: R5 (2013).
2. Li, H., *et al.* New insights on the MMP-13 regulatory network in the pathogenesis of early osteoarthritis. *Arthritis Res Ther* **19**: 248 (2017).

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

Fluorescent microplate reader capable of reading $\lambda_{exc}/\lambda_{em}=328\text{ nm}/393\text{ nm}$

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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. 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 (usually 10% DMSO in water) | 5 μ l | - | 5 μ l |
| MMP13 (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 inhibitor buffer (same solution without inhibitor; usually 10% DMSO in water) to the "Blank" and "Positive Control" wells.

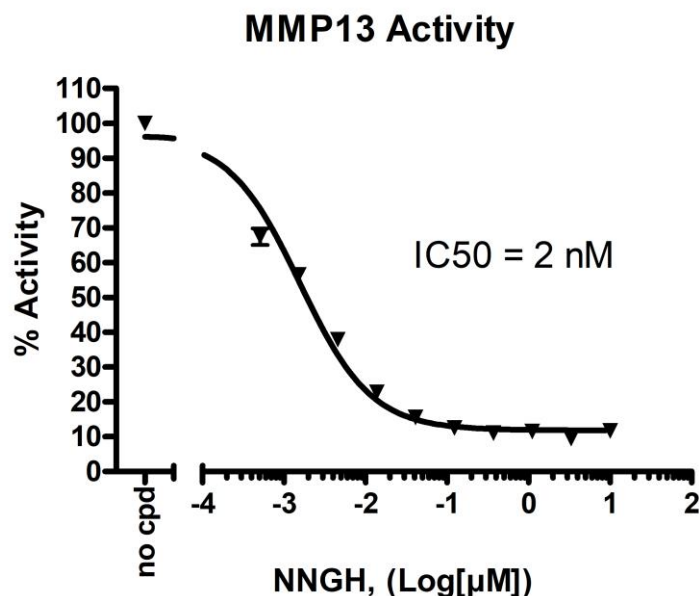
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- 6) Thaw MMP13 on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full content of the tube. Aliquot MMP13 into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C . Note: MMP13 enzyme is sensitive to freeze/thaw cycles. Do not re-use diluted enzyme.
- 7) Dilute MMP13 in 1x assay buffer at $1.6\text{ ng}/\mu\text{l}$ (32 ng per reaction).
- 8) Add $20\ \mu\text{l}$ diluted MMP13 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 MMP13 enzyme activity by NNGH, measured using the *Fluorogenic MMP13 Assay Kit* (BPS Bioscience #79991). 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. |

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