

MMP RED and GREEN Drug Discovery Kits

Featuring Sensitive, Long-wavelength Fluorogenic Substrates

The matrix metalloproteinases, or MMPs, are extracellular proteases that function at a neutral pH to cleave a wide variety of substrates. The general structure of an MMP protein comprises a pre domain to direct secretion from the cell, a pro domain, a catalytic domain, and a C-terminal hemopexin domain. The catalytic site involves a coordinately-bound zinc ion. The inactive, or zymogen, form of the enzyme is activated by disruption of one of the coordinate bonds, usually via proteolytic removal of the pro domain [4].

Substrates include basement membrane and extracellular matrix components, growth and death factors, cytokines, and cell and matrix adhesion molecules [1-3]. The broad range of substrate specificities and expression patterns of MMPs results in their involvement in many different processes, both normal and pathological. Aberrant expression has been noted in cancer, angiogenesis, arthritis, inflammation, periodontal disease, emphysema, multiple sclerosis, pre-eclampsia, and chronic wounds, among others [1-3].

MMP RED and GREEN Drug Discovery Kits

The MMP RED and GREEN Drug Discovery Kits are complete assay systems designed to screen MMP inhibitors, using the quenched fluorogenic substrate OmniMMP™ RED, or, in the case of MMP-3, MMP-3 Fluorogenic Substrate. The assays are performed in a convenient 96-well microplate format, with the compound NNGH [5] also included as a prototypic control inhibitor.

- Improved red-shifted substrate with better kinetics and brighter signal
- Includes *active* recombinant enzyme, substrate, and assay buffer
- Convenient real-time kinetics of cleavage is easily determined
- Microplate format for high-throughput screening
- Detailed instructions provided

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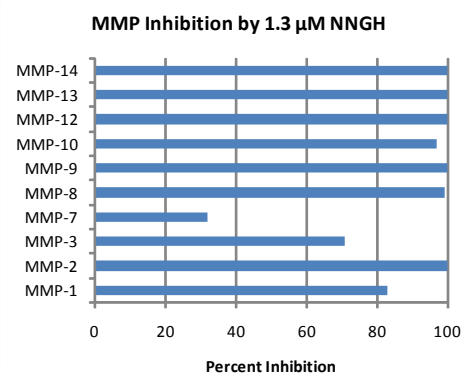


FIGURE 1: Inhibition of ten MMPs by 1.3 μM NNGH.

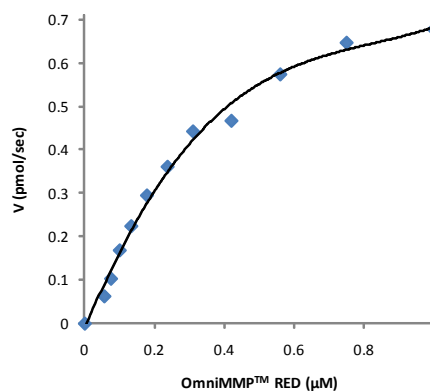


FIGURE 2: Determination of k_{cat}/K_m for MMP-1 using OmniMMP™ RED. $K_m = 0.48 \mu\text{M}$, $k_{cat}/K_m = 6.7 \times 10^6 \text{ M}^{-1}\text{sec}^{-1}$.

The **new** OmniMMP™ RED and MMP-3 Fluorogenic Substrates offer key advantages over other MMP substrates.

- 1) Emission at the higher end of the spectrum (576nm and 521nm, respectively, after excitation at 545nm and 494nm) avoids the interference at lower wavelengths often exhibited by screening compounds, and by substances commonly found in biological samples and tissue culture medium.
- 2) Highly quenched (very low background), but once cleaved by MMPs emits extremely bright signal.
- 3) MMP substrate peptides inherently display poor aqueous solubility, often with K_m s near their limits of solubility, making enzyme and inhibitor kinetics difficult. MMP K_m s for OmniMMP™ RED and MMP-3 Fluorogenic Substrates are well below the solubility limits of the substrates.
- 4) In addition to the efficient binding as exhibited by low K_m s, OmniMMP™ RED and MMP-3 Fluorogenic Substrates are avidly cleaved by MMPs, with k_{cat}/K_m s in the range of 10^5 - 10^7 M⁻¹sec⁻¹.
- 5) Better kinetics allows lower substrate concentrations, avoiding inhibition by the substrate or competition with the inhibitor at the active site.
- 6) The ultra-strong fluorescence of OmniMMP™ RED and MMP-3 Fluorogenic Substrates allows for substrate concentrations much lower than the K_m , a condition generally desirable in inhibitor screening assays

LIT: [1] Matrix metalloproteinases: they're not just for matrix anymore!: L.J. McCawley & L.M. Matrisian; Curr. Opin. Cell Biol. **13**, 534 (2001) ▪ [2] Updated biological roles for matrix metalloproteinases and new "intracellular" substrates revealed by degradomics: G.S. Butler & C.M. Overall; Biochemistry **48**, 10830 (2009) ▪ [3]. Matrix metalloproteinases: regulators of the tumor microenvironment: K. Kessenbrock & Z. Werb; Cell **141**, 52 (2010) ▪ [4] J.F. Woessner & H. Nagase Metalloproteinases and TIMPs. 2000 Oxford University Press. 5. Discovery of CGS 27023A, a non-peptidic, potent, and orally active stromelysin inhibitor that blocks cartilage degradation in rabbits: L.J. MacPherson et al.; J. Med. Chem. **40**, 2525 (1997).

MMP RED and GREEN Drug Discovery Kits

Product	Prod. No.	Size
MMP-1 fluorimetric drug discovery kit, RED	BML-AK301-0001	96 wells
MMP-2 fluorimetric drug discovery kit, RED	BML-AK302-0001	96 wells
MMP-3 fluorimetric drug discovery kit, GREEN	BML-AK303-0001	96 wells
MMP-7 fluorimetric drug discovery kit, RED	BML-AK304-0001	96 wells
MMP-8 fluorimetric drug discovery kit, RED	BML-AK305-0001	96 wells
MMP-9 fluorimetric drug discovery kit, RED	BML-AK306-0001	96 wells
MMP-19 fluorimetric drug discovery kit, RED	BML-AK307-0001	96 wells
MMP inhibitor profiling kit, fluorimetric RED	BML-AK308-0001	96 wells

Related Products

Product	Prod. No.	Size
OmniMMP™ RED fluorogenic substrate	BML-P277-0100	0.1 mg
MMP-3 fluorogenic substrate	BML-P278-0100	0.1 mg

For MMP enzymes and antibodies please visit www.enzolifesciences.com

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