



MMP Inhibitor Profiling Kit, Fluorometric

Designed to examine the specificity of inhibitors against a panel of ten matrix metalloproteinase enzymes, using a quenched fluorogenic peptide.

Instruction Manual

BML-AK016

For research use only



USE FOR RESEARCH PURPOSES ONLY

Unless otherwise specified expressly on the packaging, all products sold hereunder are intended for and may be used for research purposes only and may not be used for food, drug, cosmetic or household use or for the diagnosis or treatment of human beings. Purchase does not include any right or license to use, develop or otherwise exploit these products commercially. Any commercial use, development or exploitation of these products or development using these products without the express written authorization of Enzo Life Sciences, Inc. is strictly prohibited. Buyer assumes all risk and liability for the use and/or results obtained by the use of the products covered by this invoice whether used singularly or in combination with other products.

LIMITED WARRANTY; DISCLAIMER OF WARRANTIES

These products are offered under a limited warranty. The products are guaranteed to meet all appropriate specifications described in the package insert at the time of shipment. Enzo Life Sciences' sole obligation is to replace the product to the extent of the purchasing price. All claims must be made to Enzo Life Sciences, Inc., within five (5) days of receipt of order. THIS WARRANTY IS EXPRESSLY IN LIEU OF ANY OTHER WARRANTIES OR LIABILITIES, EXPRESS OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, AND NON- INFRINGEMENT OF THE PATENT OR OTHER INTELLECTUAL PROPERTY RIGHTS OF OTHERS, AND ALL SUCH WARRANTIES (AND ANY OTHER WARRANTIES IMPLIED BY LAW) ARE EXPRESSLY DISCLAIMED.

TRADEMARKS AND PATENTS

Several Enzo Life Sciences products and product applications are covered by US and foreign patents and patents pending. Enzo is a trademark of Enzo Life Sciences, Inc.

**FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

✦ MMP Inhibitor Profiling Kit, Fluorimetric – BML-AK016 ✦**BACKGROUND**

The matrix metalloproteinases, or MMPs, are extracellular proteases that function at a neutral pH to cleave a wide variety of substrates. These include basement membrane and extracellular matrix components, growth and death factors, cytokines, and cell and matrix adhesion molecules^{1,2,3}. 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^{1,2,4}. The general structure of an MMP protein consists of 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 domain⁵.

The *MMP Inhibitor Profiling Kit* is a complete assay system designed to examine the specificity of inhibitors against a panel of ten matrix metalloproteinase enzymes, using a quenched fluorogenic peptide: OmniMMP™ fluorogenic substrate Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH₂ [Mca=(7-methoxycoumarin-4-yl)-acetyl; Dpa=N-3-(2,4-dinitrophenyl)-L- α - β -diaminopropionyl]⁶. Mca fluorescence is quenched by the Dpa group until cleavage by MMPs at the Gly-Leu bond separates the two moieties^{6,7}. The assays are performed in a convenient 96-well microplate format. The compound NNGH⁸ is also included as a prototypic control inhibitor.

Please contact Enzo Life Sciences for kit components in bulk.

REFERENCES:

1. G. Bergers and L.M. Coussens *Curr. Opin. Genet. Dev.* 2000 **10** 120
2. S.D. Shapiro *Curr. Opin. Cell Biol.* 1998 **10** 602
3. V. Noe *et al. J. Cell Sci.* 2000 **114** 111
4. A.R. Nelson *et al. J. Clin. Oncol.* 2000 **18** 1135
5. J.F. Woessner and H. Nagase *Metalloproteinases and TIMPs.* 2000 Oxford University Press
6. C.G. Knight *et al. FEBS Lett.* 1992 **296** 263
7. M.M. Smith *et al. J. Biol. Chem.* 1995 **270** 6440
8. L.J. MacPherson *et al. J. Med. Chem.* 1997 **40** 2525

PLEASE READ ENTIRE BOOKLET BEFORE PROCEEDING WITH THE ASSAY. CAREFULLY NOTE THE HANDLING AND STORAGE CONDITIONS OF EACH KIT COMPONENT. PLEASE CONTACT ENZO LIFE SCIENCES TECHNICAL SERVICES FOR ASSISTANCE IF NECESSARY.

COMPONENTS OF BML-AK016 KIT**BML-SE180-9090 MMP-1 ENZYME (HUMAN, RECOMBINANT)**

FORM: Recombinant human MMP-1 catalytic domain (19.9kDa); 30.6U/μL.
One U=100 pmol/min@ 37°C, 100 μM thiopeptide P125.

STORAGE: -70°C; Avoid freeze/thaw cycles

QUANTITY: 2000U

BML-SE237-9090 MMP-2 ENZYME (HUMAN, RECOMBINANT)

FORM: Recombinant human MMP-2 catalytic domain (40kDa); 3.28 U/μL.
One U=100 pmol/min@ 37°C, 100 μM thiopeptide.

STORAGE: -70°C; Avoid freeze/thaw cycles

QUANTITY: 150U

BML-SE109-9090 MMP-3 ENZYME (HUMAN, RECOMBINANT)

FORM: Recombinant human MMP-3 catalytic domain (19.5kDa); 10 U/μL.
One U=100 pmol/min@ 37°C, 100 μM thiopeptide.

STORAGE: -70°C; Avoid freeze/thaw cycles

QUANTITY: 300U

BML-SE181-9090 MMP-7 ENZYME (HUMAN, RECOMBINANT)

FORM: Recombinant human MMP-7 catalytic domain (20.4kDa); 4.5 U/μL.
One U=100 pmol/min@ 37°C, 100 μM thiopeptide.

STORAGE: -70°C; Avoid freeze/thaw cycles

QUANTITY: 150U

BML-SE255-9090 MMP-8 ENZYME (HUMAN, RECOMBINANT)

FORM: Recombinant human MMP-8 catalytic domain (20.3kDa); 9.2 U/μL.
One U=100 pmol/min@ 37°C, 100 μM thiopeptide.

STORAGE: -70°C; Avoid freeze/thaw cycles

QUANTITY: 200U

BML-SE360-9090 MMP-9 ENZYME (HUMAN, RECOMBINANT)

FORM: Recombinant human MMP-9 catalytic domain (39kDa); 2.68 U/μL. One U=100 pmol/min@ 37°C, 100 μM thiopeptide.

STORAGE: -70°C; Avoid freeze/thaw cycles

QUANTITY: 130U

BML-SE329-9090 MMP-10 ENZYME (HUMAN, RECOMBINANT)

FORM: Recombinant human MMP-10 catalytic domain (19.4kDa); 5 U/μL.
One U=100 pmol/min@ 37°C, 100 μM thiopeptide.

STORAGE: -70°C; Avoid freeze/thaw cycles

QUANTITY: 150U

BML-SE138-9090 MMP-12 ENZYME (HUMAN, RECOMBINANT)

FORM: Recombinant human MMP-12 catalytic domain (20.3kDa); 10U/μL.
One U=100 pmol/min@ 37°C, 100 μM thiopeptide.

STORAGE: -70°C; Avoid freeze/thaw cycles

QUANTITY: 140U

BML-SE246-9090 MMP-13 ENZYME (HUMAN, RECOMBINANT)**FORM:** Recombinant human MMP-13 catalytic domain (20.4kDa); 3.45 U/ μ L.One U=100 pmol/min@ 37°C, 100 μ M thiopeptide.**STORAGE:** -70°C; Avoid freeze/thaw cycles**QUANTITY:** 180U**BML-SE259-9090 MMP-14 ENZYME (HUMAN, RECOMBINANT)****FORM:** Recombinant human MMP-14 catalytic domain (22.5kDa); 12U/ μ L.One U=100 pmol/min@ 37°C, 100 μ M thiopeptide.**STORAGE:** -70°C; Avoid freeze/thaw cycles**QUANTITY:** 300U**BML-P126-9090 SUBSTRATE (OmniMMP™ fluorogenic substrate peptide; MW=1093.2)****FORM:** 400 μ M (437 μ g/mL) in DMSO**STORAGE:** -70°C**QUANTITY:** 200 μ L**BML-P127-9090 CALIBRATION STANDARD (OmniMMP™ fluorogenic control peptide, MCA-Pro-Leu-OH; MW=444.5)****FORM:** 40 μ M (17.8 μ g/mL) in DMSO**STORAGE:** -70°C**QUANTITY:** 50 μ L**BML-PI115-9090 INHIBITOR (NNGH; MW=316.4)****FORM:** 1.3 mM in DMSO**STORAGE:** -70°C**QUANTITY:** 50 μ L**BML-KI175-0020 ASSAY BUFFER**50 mM HEPES, 10 mM CaCl₂, 0.05% Brij-35, pH 7.5**FORM:** Liquid in screw-cap plastic bottle**STORAGE:** Room temperature**QUANTITY:** 2 x 20 mL**80-2406 96-WELL WHITE NBS MICROPLATE****STORAGE:** Room temperature.

OTHER MATERIALS REQUIRED

- Fluorescent microplate reader capable of excitation at 328 nm and emission at 420 nm. The following Ex/Em have also been used: 320,340/393,400,405 nm.
- Pipetmen or multi-channel pipetmen capable of pipetting 1-100 μ L accurately.
- Ice bucket to keep reagents cold until use.
- Water bath or incubator for component temperature equilibration.

EXPERIMENTAL METHODS

Note on storage: *Store all components except the microplate (room temperature) at -70°C for the highest stability. Components with storage temperatures other than -70°C can be stored at the temperature listed OR at -70°C . The MMP enzymes should be handled carefully in order to retain maximal enzymatic activity. They are stable, in diluted or concentrated form, for several hours on ice. As supplied, the MMP enzymes are stable for at least 5 freeze/thaw cycles. If necessary, to minimize the number of freeze/thaw cycles, aliquot the MMPs into separate tubes and store at -70°C . When setting up the assay, do not maintain diluted components at reaction temperature (e.g. 37°C) for an extended period of time prior to running the assay.*

To start assay:

1. Briefly warm kit components BML-P126-9090, BML-P127-9090, and BML-PI115-9090 to RT to thaw DMSO.
2. Dilute control inhibitor (NNGH, BML-PI115-9090) 1/200 in assay buffer BML-KI175 as follows. Add 1 μ L inhibitor into 200 μ L assay buffer, in a separate tube. Warm to reaction temperature (e.g. 37°C).
3. Thaw the DMSO stock vial of substrate BML-P126-9090 and dilute sufficient volume to 40 μ M in assay buffer (10 μ l needed per well). Warm to reaction temperature (e.g. 37°C).
4. Shortly before assay, dilute MMP enzymes in assay buffer to required total volume (20 μ l are needed per well), using the following ratios for dilution:
 - MMP-1: 1/40 MMP-9: 1/60
 - MMP-2: 1/70 MMP-10: 1/100
 - MMP-3: 1/70 MMP-12: 1/285
 - MMP-7: 1/70 MMP-13: 1/50
 - MMP-8: 1/100 MMP-14: 1/100

Note: MMP-3 is unique in that its pH optimum is 6.0. Activity of this enzyme in pH 7.5 buffer is somewhat reduced; thus the dilution for MMP-3 has been adjusted accordingly.

Warm to reaction temperature (e.g. 37°C).

5. Pipet assay buffer into each desired well of the 1/2 volume microplate as follows:

- Calibration = 80 μ L in 3 wells (see step 11)
- Control (no inhibitor) = 70 μ L
- Inhibitor NNGH = 50 μ L
- Test inhibitor = varies (see Table 1)

Example of plate: well# sample

- A1 Calibration
- B1 Calibration
- C1 Calibration
- D1 Control/MMP-1
- E1 Control/MMP-1
- F1 Inhibitor NNGH/MMP-1
- G1 Inhibitor NNGHMMP-1
- H1 Test inhibitor/MMP-1
- A2 Test inhibitor/MMP-1
- B2 Control/MMP-2
- C2 Control/MMP-2
- D2 Test inhibitor/MMP-2
- E2 Test inhibitor/MMP-2...

6. Allow microplate to equilibrate to assay temperature (e.g. 37°C).
7. Add 20 μ L MMP (diluted in step 4) to the control, inhibitor NNGH, and test inhibitor wells.
8. Add 20 μ L NNGH inhibitor (diluted in step 2) to the inhibitor NNGH wells only. Final inhibitor concentration = 1.3 μ M. See figure (below) for example of inhibition of MMPs by NNGH.
9. Add desired volume of test inhibitor to appropriate wells. See Table 1.
10. Incubate plate for 30-60 minutes at reaction temperature (e.g. 37°C) to allow inhibitor/enzyme interaction.
11. In the meantime, calibrate the fluorescent microplate reader, using Ex/Em=328/420: Prewarm assay buffer to reaction temperature in 3 wells in the microplate, then to each add 10 μ L BML-P126-9090 substrate

peptide to give the concentration to be used in the assay (e.g., for 4 μM final add 10 μL 40 μM) and mix. When the fluorescent signal is constant, use this reading as the zero (Blank) value in arbitrary fluorescence units (RFUs). Using the same wells, with their mixtures of substrate peptide and buffer, add 10 μL calibration standard peptide BML-P127-9090 to give 3 different final molar concentrations ranging between 2 and 10% of the substrate peptide molar concentration (e.g., 80, 200, and 400nM) and measure their fluorescence. Use these values to build a standard curve relating micromolar BML-P127-9090 concentration (x axis) to RFUs (y axis). The slope of the line is the conversion factor (CF). If multiple concentrations of substrate peptide are used, such as in kinetic determinations, step 11 must be performed for each concentration, due to absorptive quenching by the substrate peptide. Note: this calibration can be done at any time.

12. Start reactions by the addition of 10 μL BML-P126-9090 substrate (diluted and equilibrated to reaction temperature in step 3). Final substrate concentration = 4 μM .
13. Continuously read plates in the fluorescent microplate reader, using Ex/Em=328/420. For example, record data at 1 minute time intervals for 10 minutes.
14. Perform data analysis (see below).

NOTE: Retain microplate for future use of unused wells.

TABLE 1. Example of Samples.

Sample	Assay buffer	MMP	Inhibitor (6.5 μM)	Substrate (40 μM)	Total Volume
Control	70 μL	20 μL	0	10 μL	100 μL
Inhibitor NNGH	50 μL	20 μL	20 μL	10 μL	100 μL
Test inhibitor*	X μL	20 μL	Y μL	10 μL	100 μL

*Test inhibitor is the experimental inhibitor. Dissolve/dilute inhibitor into assay buffer and add to appropriate wells at desired volume “Y”. Adjust volume “X” to bring the total volume to 100 μL .

DATA ANALYSIS

Plotting

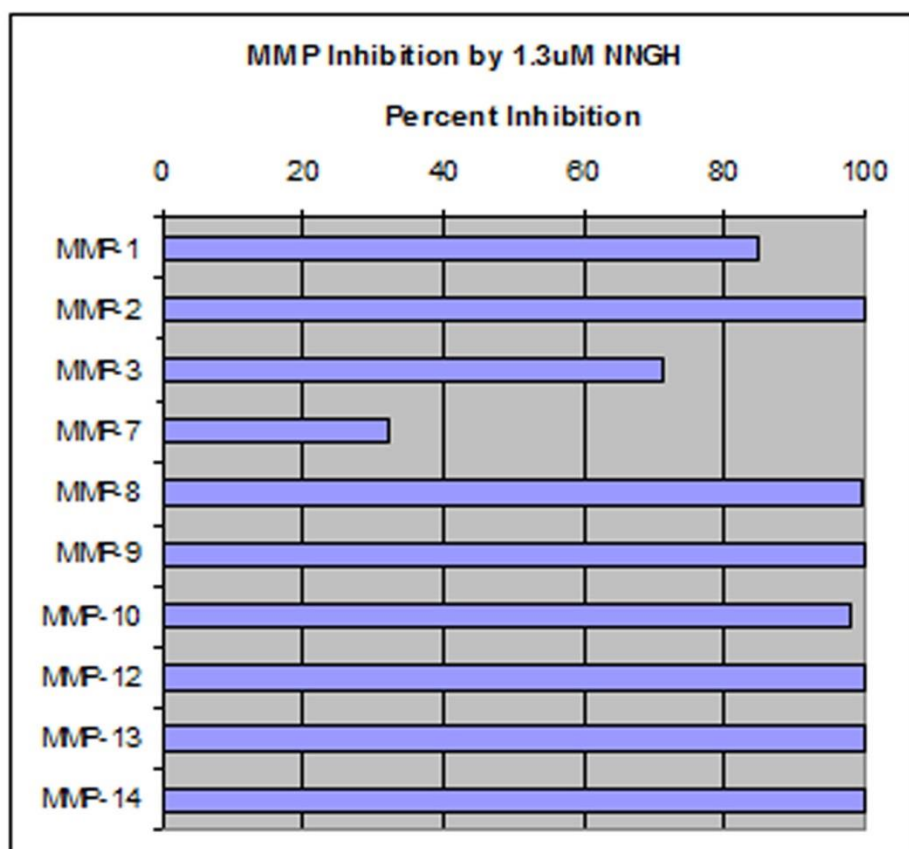
15. Plot data as RFUs (minus Blank RFU value determined during calibration, step 11) versus time for each sample.
16. Determine the range of initial time points during which the reaction is linear.
17. Obtain the initial reaction velocity (**V**) in RFUs/min: determine the slope of a line fit to the initial linear portion of the data plot using an appropriate routine.
18. It is best to use a range of inhibitor concentrations, each in duplicate. Average the slopes of duplicate samples.

Data Reduction

To determine inhibitor % remaining activity:

Inhibitor % activity remaining = (**V** inhibitor / **V** control) x 100

See figure for example of results.



To determine the activity of the samples expressed as picomoles substrate hydrolyzed per minute:

$$X \text{ pmoles substrate/min} = 1/CF \times V \times \text{vol}$$

Where CF is the conversion factor (micromolar concentration/RFUs, from step 11), **V** is initial reaction velocity (RFUs/min, from step 17), and vol is the reaction volume in microliters (100).



Product Manual

Notes



Product Manual

Notes



Product Manual

GLOBAL HEADQUARTERS

Enzo Life Sciences Inc.
10 Executive Boulevard
Farmingdale, NY 11735
Toll-Free: 1.800.942.0430
Phone: 631.694.7070
Fax: 631.694.7501
info-usa@enzolifesciences.com

EUROPE/ASIA

Enzo Life Sciences (ELS) AG
Industriestrasse 17
CH-4415 Lausen
Switzerland
Phone: +41/0 61 926 89 89
Fax: +41/0 61 926 89 79
info-ch@enzolifesciences.com

For local distributors and detailed product information visit us online:
www.enzolifesciences.com