PROTOCOL

Guidelines for use of MMP-3 fluorogenic substrate (Enzo Life Sciences Cat. # BML-P278)

Dissolve the peptide with DMSO for a 1 mM stock solution. To ensure accurate concentration is achieved when dissolving peptides, both peptide purity and content need to be taken into account. Here is an example calculating the amount of DMSO needed to dissolve 0.1 mg Cat. # BML-P278 to 1 mM, when its purity is 96% and content is 75%: (mol/2156.9 g)x($1x10^3$ mmol/mol)x(L/1 mmol)x($1x10^6$ µl/L)x(g/$1x10^3$ mg)x(0.75x0.1 mg)x(0.96)=33.4 µl DMSO. Store at –20°C in aliquots; DMSO stocks are stable for 3-6 months at -20°C.

1. Prepare 10X assay buffer:
   500mM MES (2-[N-morpholino]ethane-sulfonic acid)
   100mM $CaCl_2$
   0.5% Brij-35
   pH to 6.0

2. Because optimal amounts of peptide and MMP will vary, it is best to initially use a range of both. Suggested concentration ranges are 0.1-1 µM peptide (depending on application, enzyme, and other factors), and 0-50nM MMP. In the end, initial velocity should be linear with respect to enzyme concentration, and the peptide concentration must be well below K_m for continuous assays or above K_m for endpoint assays. NOTE: vigorously mix intermediate aqueous dilutions immediately prior to use, as aqueous solubility is limited, but DMSO, which inhibits MMP activity, must be kept below 1% in the assay.

3. To calibrate the fluorometer, it is recommended that a fluorogenic control (5'-FAM, Cat. #BML-KI583) be used in the following manner: Prewarm assay buffer to the reaction temperature in 2 or 3 wells in the microplate, then add the substrate peptide #BML-P278 to the concentration to be used in the assay, and mix. Once the fluorescent signal is constant, use this reading as the zero value. Using the same wells, with their mixtures of substrate peptide and buffer, add control #BML-KI583 at 2 or 3 final molar concentrations ranging between 2 and 10% of the substrate peptide molar concentration, such that the volume in the well matches that of the assay to be run. Read fluorescence. Use these values to build a standard curve relating fluorescence intensity to concentration of #BML-KI583. If multiple concentrations of substrate peptide are used, such as in K_m determinations, this procedure must be performed for each substrate concentration, due to absorptive quenching by the substrate peptide.

4. Reactions (diluted assay buffer, enzyme, and substrate) are monitored in a fluorometer using an exitation filter of 494 nm and detection filter of 521 nm (with cutoff, if available, set at 515 nm) at 25-37°C. Either cuvette or microplate (using white flat-bottomed microplates) format can be used. The reaction can be stopped with 50mM EDTA if desired. If MMP inhibitors are being used, incubate MMP in buffer with inhibitor for 30-60 min. prior to assay.

NOTE: This serves as a guide only. Exact assay conditions must be determined by the user.

Related products also available from Enzo Life Sciences include recombinant and purified MMPs, MMP inhibitors, and MMP inhibitor screening kits.