Assay Method Using Nma/Dnp type Fluorescence-Quenching Substrates

Principle

The highly fluorescent 2-(N-methylamino)benzoyl (Nma) group in the substrates such as Code 3217 and 3224 is efficiently quenched by the 2,4-dinitrophenyl (Dnp) group. When an enzyme of interest cleaves any peptide bond between Nma- and Dnp-containing amino acid residues in the substrate, the fluorescence at λex = 340 nm and λem = 440 nm increases in proportion to the release of the Nma fluorophore from the internal Dnp quencher.

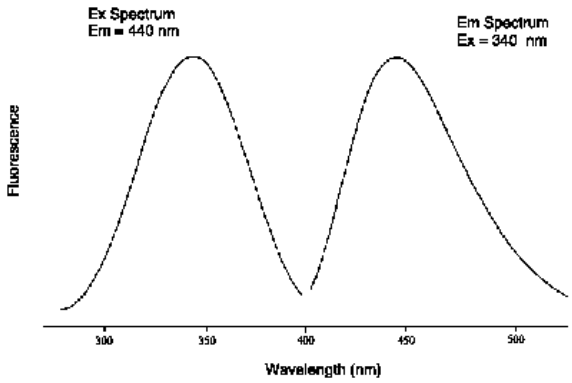


Fig. Fluorescence Spectra of a 1:1 mixture of D-Arg(Nma)-Gly and Ala-Phe-Pro-Lys(Dnp)-D-Arg-D-Arg

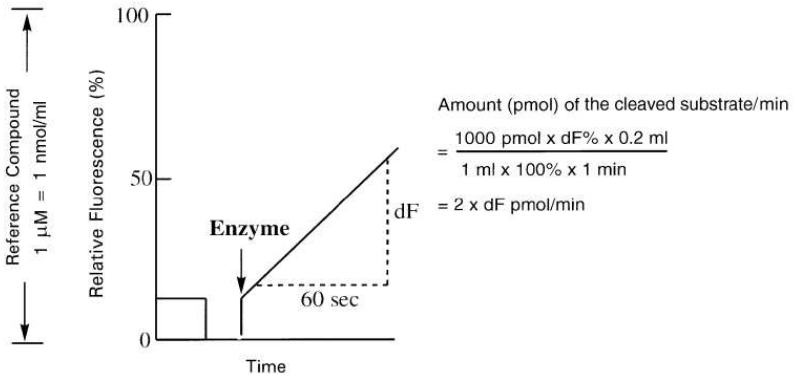
Reagents

1) Substrate stock solution: 100 μM^{-10} µM in an appropriate solvent
2) Reference compounds stock solution: a 1:1 mixture of two solutions of Code 3720-v and 3721-v, each of which is reconstituted by dissolving peptides in 0.5 ml of DMSO at the concentration of 2 mM (1 mM, each reference compound)
3) Enzyme solution: an enzyme of interest in an appropriate buffer
4) Buffer

Procedure

Choose the proper conditions for the measurement, such as substrate concentration and sensitivity setting, depending on the purpose of the experiment and the instrument available. Described here is one of the recommended procedures for the fluorometric method (initial-rate method).

1) Set a fluorescence spectrometer at λex = 340 nm and λem = 440 nm (1.0 Relative fluorescence unit at 1 µM of the reference compound)
2) Pipette 160 µl of buffer and 2-20 µl of substrate solution in well for final concentration of 1 µM.
3) Incubate in the fluorescence spectrophotometer for 3-4 min for temperature equilibration
4) Add 20 µl of enzyme solution prepared at an appropriate concentration
5) Record the increase of the fluorescence intensity
6) Calculate the amount of the cleaved substrate using the following equation



1) D.M. Bickett, M.D. Green, J. Berman, M. Dezube, A.S. Howe, P.J. Brown, J.T. Roth and G.M. McGeehan, Anal. Biochem., 212, 58 (1993).
2) S. Tanskul, K. Oda, H. Oyama, N. Noparatnaraporn, M. Tsunemi and K. Takada, Biochem. Biophys. Res. Commun., 309, 547 (2003).