

Assay Method Using MOCAC/Dnp type Fluorescence-Quenching Substrates (Example using Code 3163-v MOCAC-Pro-Leu-Gly-Leu-A₂pr(Dnp)-Ala-Arg-NH₂)

Principle

The highly fluorescent (7-methoxycoumarin-4-yl)acetyl (MOCAC) group in the substrate such as Code 3163 is efficiently quenched by the 2,4-dinitrophenyl (Dnp)group. When metalloenzyme cleaves to the Gly-Leu bond, the fluorescence at $\lambda_{ex} = 328 \text{ nm}$ and $\lambda_{em} = 393 \text{ nm}$ increases 190-fold.

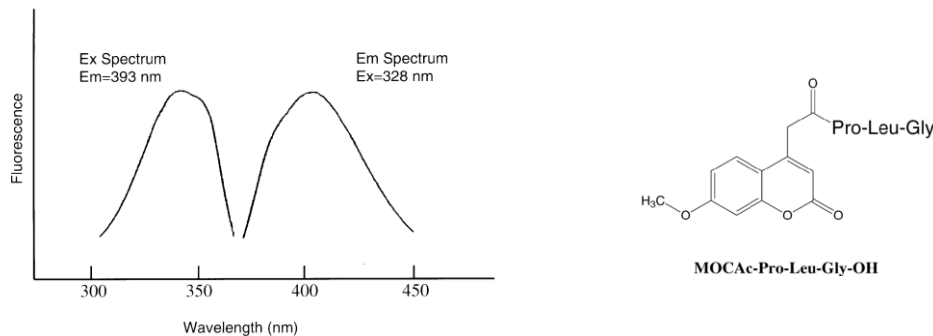


Fig. Fluorescence Spectra of MOCAC-Pro-Leu-Gly-OH

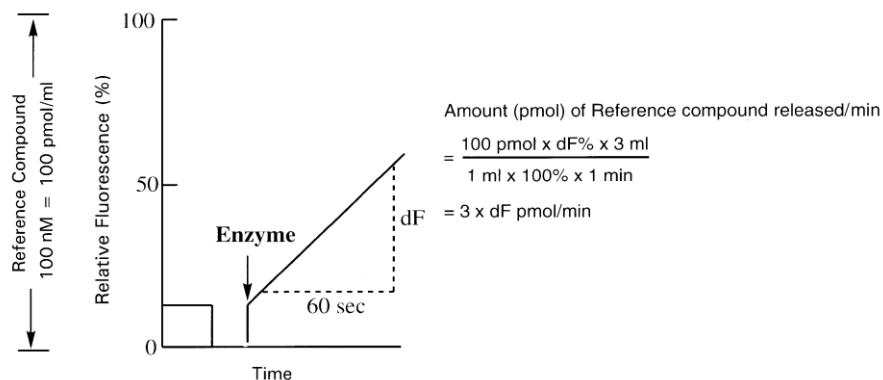
Reagents

1. Substrate stock solution: Code 3163-v in DMSO at $2 \times 10^{-4} \text{ M}$
2. MOCAC-Pro-Leu-Gly (reference compound) stock solution: Code 3164-s in 10 ml DMSO ($2 \times 10^{-5} \text{ M}$)
3. Buffer: 0.1 M-Tris HCl, pH 7.5 containing 0.1 M NaCl, 10 mM CaCl₂ and 0.05% Brij-35
4. Enzyme solution

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 spectrophotometer at $\lambda_{ex} = 328 \text{ nm}$ and $\lambda_{em} = 393 \text{ nm}$ at 25°C (1.0 Relative fluorescence unit at 10^{-7} M of the reference compound)
- 2) Pipette 2900 μl of buffer and 50 μl of substrate stock solution into the cuvette
- 3) Incubate in the fluorescence spectrophotometer for 3-4 min (for temperature equilibration)
- 4) Add 50 μl of enzyme solution
- 5) Record the increase of the fluorescence intensity for 3-4 min
- 6) Calculate the amount of reference compound released using the following equation



1) C.G. Knight, F. Willenbrock, and G. Murphy, *FEBS Lett.*, 296, 263 (1992).