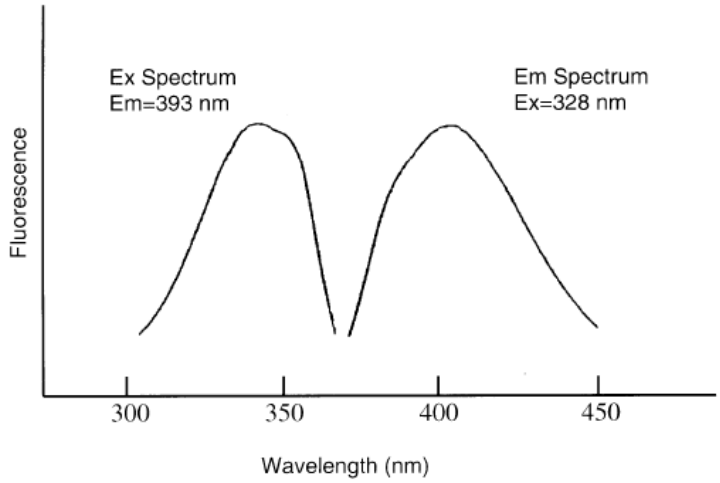
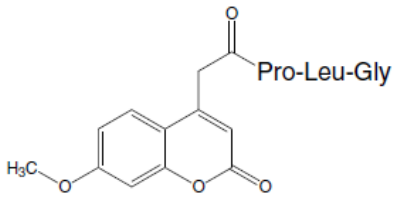
Assay Method Using MOCAc/Dnp type Fluorescence-Quenching Substrates

(Example using Code 3163-v MOCAc-Pro-Leu-Gly-Leu-A_2pr(Dnp)-Ala-Arg-NH_2)

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 λex = 328 nm and λem = 393 nm increases 190-fold [1].





MOCAc-Pro-Leu-Gly-OH

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

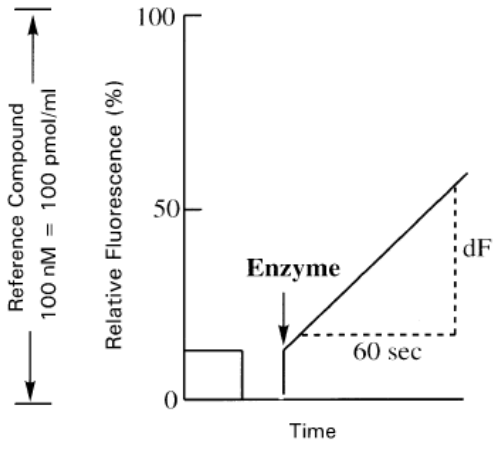
Reagents

1) Substrate stock solution: Code 3163-v in DMSO at 2 × 10^{-4} M
2) MOCAc-Pro-Leu-Gly (reference compound) stock solution: Code 3164-s in 10 ml of DMSO (2 × 10^{-5} M)
3) Buffer: 0.1 M-Tris • HCl, pH 7.5 containing 0.1 M NaCl, 10 mM $CaCl_2$ 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 λex = 328 nm and λem = 393 nm at 25℃ (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



$$\text{Amount (pmol) of Reference compound released/min} = \frac{100 \text{ pmol} \times dF\% \times 3 \text{ ml}}{1 \text{ ml} \times 100\% \times 1 \text{ min}} = 3 \times dF \text{ pmol/min}$$

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

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