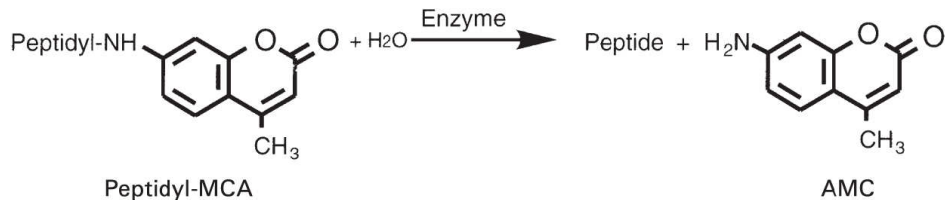


Assay Methods Using Peptidyl-AMC (MCA) Substrates (2) (Measurement on an Auto-Fluorescence Spectrophotometer for Multiplate)

Principle



Reagents

1. Substrate stock solution: Vial, in DMSO at 10 mM
2. AMC stock solution: Content of vial (Code 3099-vAMC), in DMSO at 1 mM
3. Buffer
4. Enzyme solution

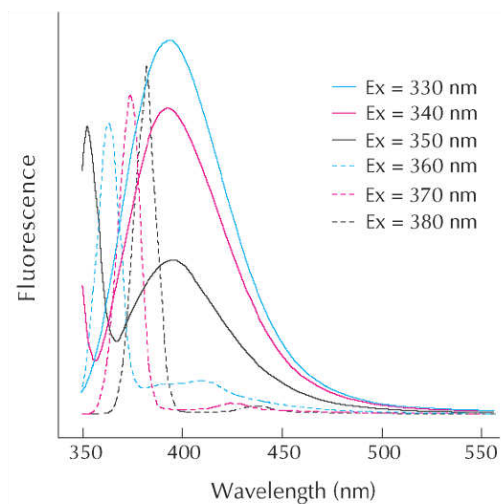


Fig. 2a Fluorescence Spectra of Peptidyl-MCA (Substrate)

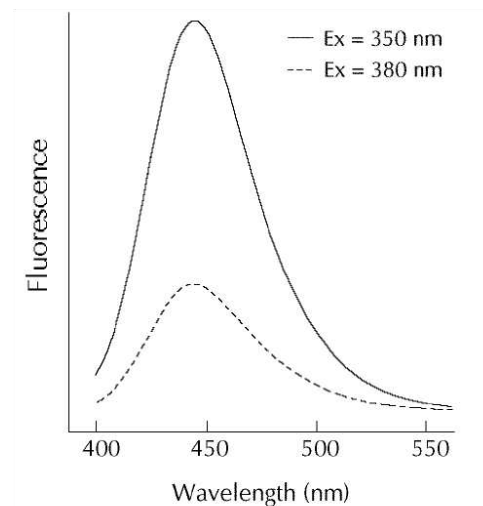


Fig. 2b Fluorescence Spectra of AMC (Product)

Procedure

Choose the proper conditions for the measurement, such as substrate, enzyme concentration and other reaction conditions, depending on the purpose of the experiment.

1. Set the auto-fluorescence spectrophotometer at $\lambda_{\text{ex}} = 380 \text{ nm}$ and $\lambda_{\text{em}} = 460 \text{ nm}$ at 25 °C (1.0 Relative fluorescence unit at 10^{-6} M of AMC)
2. Pipette 160 μl of buffer and 20 μl of substrate solution in well for final concentration of 100 μM
3. Incubate in the plate in the fluorescence spectrophotometer for 3-4 min (for temperature equilibration)
4. Take the multiplate out and add 20 μl of enzyme solution in each well
5. Mount the plate in the fluorescence spectrometer
5. Record the increase of the fluorescence intensity for 30 min with a premixing time of 3 sec
6. Calculate the amount of released AMC