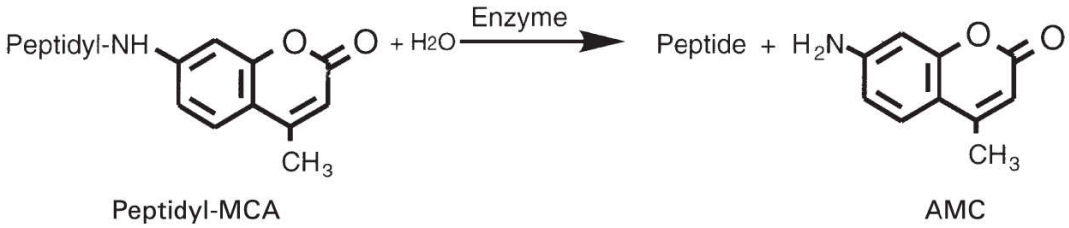
Assay Methods Using Peptidyl-AMC (MCA) Substrates (1)

Principle



Peptidyl-MCA AMC

The initial rate of increase in the AMC concentration can be monitored 1) fluorometrically at λex = 380 nm and λem = 460 nm (Fig. 1a) or 2) photometrically at 370 nm (Fig. 1b).

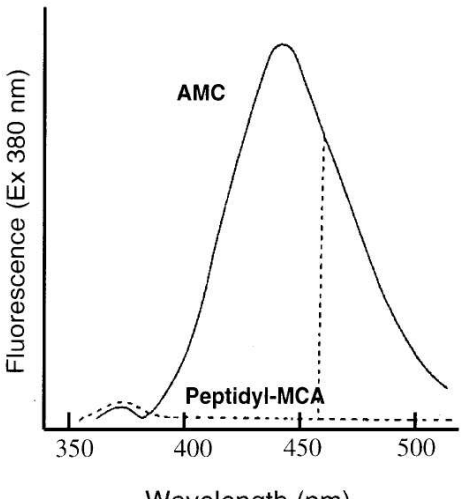


Fig. 1a Fluorescence Spectra

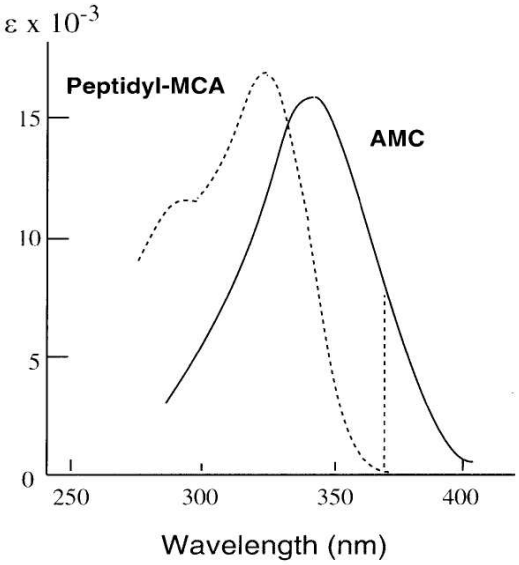


Fig. 1b UV-Absorption Spectra

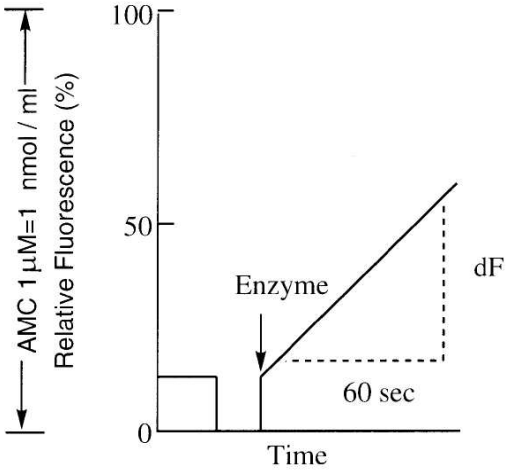
Reagents

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

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 = 380 nm and λem = 460 nm at 25 °C (1.0 Relative fluorescence unit at 10^{-6} M of AMC)
2. Pipette 2940 µl of buffer and 30 µl of substrate stock solution into the cuvette
3. Incubate in the fluorescence spectrophotometer for 3-4 min (for temperature equilibration)
4. Add 30 µl of enzyme solution
5. Record the increase of the fluorescence intensity for 3-4 min
6. Calculate the amount of AM C released using the following equation



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

$$= 0.03 \times \text{dF nmol/min}$$

* Photometric measurement can be carried out by the same procedure as that of the fluorometric method using a UV spectrophotometer. Set the wavelength at 370 nm (ε 7700).