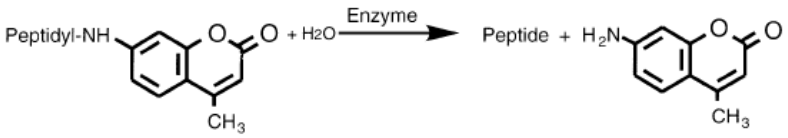
Assay Methods Using Peptidyl-MCA Substrates (1)

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).

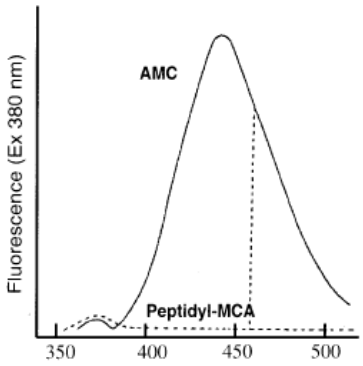


Fig. 1a Fluorescence Spectra

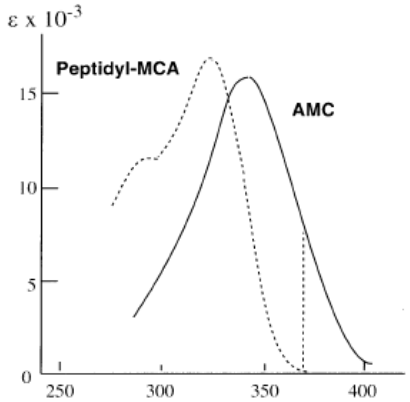


Fig. 1b UV-Absorption Spectra

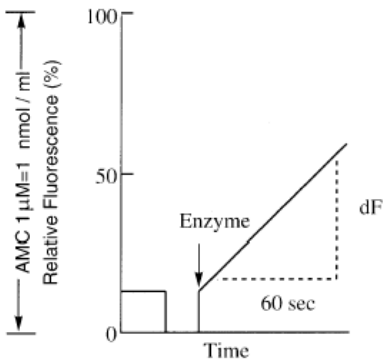
Reagents

1) Substrate stock solution: Vial, in DMSO at 10 mM
2) AMC stock solution: Content of vial (Code 3099-v AMC), 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 ℃ (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 AMC 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).

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

Reagents

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

Instrument: **Auto-fluorescence spectrophotometer**

Selection of filter:

380 nm, 390 nm or 355 nm filter will be recommended for measurement.

In using 355 nm filter, observe caution for overlapping of fluorescence of the substrate itself.

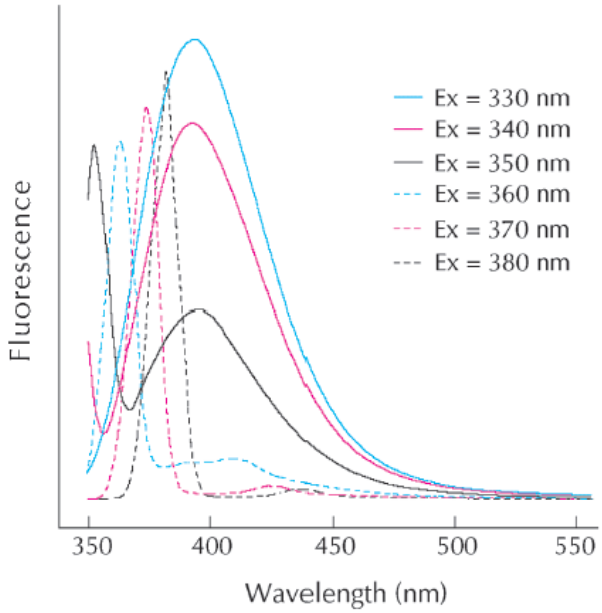


Fig. 2a Fluorescence Spectra of Peptidyl-MCA (Substrate)

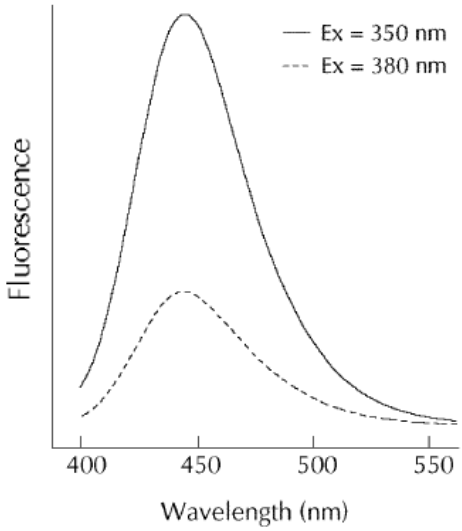


Fig. 2b Fluorescence Spectra of AMC (Product)

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 λex = 380 nm, λem = 460 nm at 25 ℃
   (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 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 spectrophotometer
6) Record the increase in fluorescence intensity for 30 min with a premixing time of 3 sec
7) Calculate the amount of released AMC

Assay Methods Using Peptidyl-MCA Substrates (3)

(Example for Caspase-7 using an Auto-fluorescence Spectrophotometer for Multiplate)

Reagents

1) Substrate stock solution: Content of vial (Code 3171-v Ac-DEVD-MCA), in DMSO at 10 mM
2) AMC standard solution: Content of vial (Code 3099-v AMC), in DMSO at 1 mM
3) Buffer: ICE standard buffer (100 mM HEPES-KOH, pH 7.5, 10% sucrose (w/v), 0.1% CHAPS (w/v) , 10 mM DTT, 0.1 mg/ml ovalbumin)
4) Enzyme solution: rec Caspase-7 in buffer

Insutrument: **Fluoroskan Ascent (Labsystems)**

This instrument can be used for both initial rate assay and end point assay

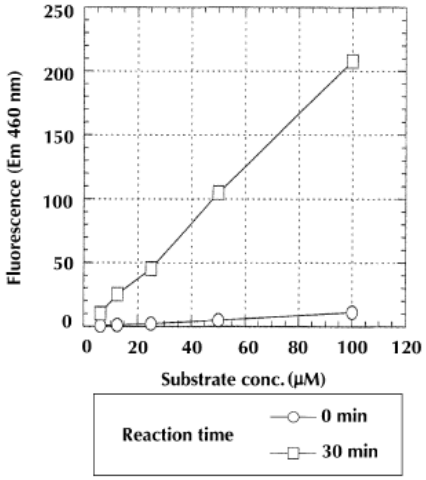
Procedure

a) Initial rate assay for 30 min at λex = 390 nm, λem = 460 nm

b) End-point (30 min) assay at 5 different substrate concentrations at λex = 355 nm or 380 nm, λem = 460 nm

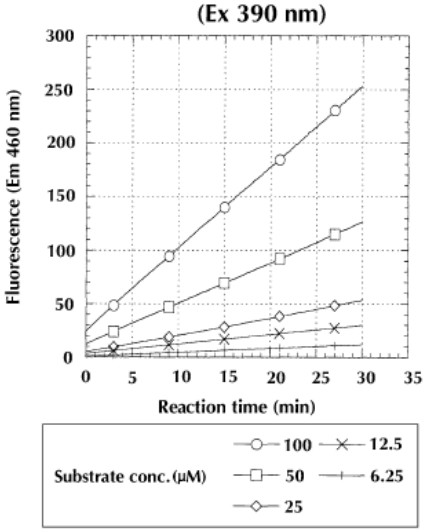
1) Set a fluorescence spectrophotometer at λex = 390 nm (or 380 nm, 355 nm), λem = 460 nm. Relative fluorescence is determined with 10^{-6} M of AMC at each condition
2) Substrate: Dilute the stock solution with the buffer (×10, ×20, ×40, ×80, ×160)
3) Caspase-7: Dissolve in buffer
4) Pipette 160 µl of buffer and 20 µl of substrate solutions (1, 1/2, 1/4, 1/8, 1/16 mM) in each well
5) Incubate in the fluorescence spectrophotometer for 3-4 min for temperature equilibration
6) Take the multiplate out and add 20 µl of enzyme solution to each well
7) Mount the plate in the fluorescence spectrophotometer
8) Calculate the amount of released AMC

b1) End-point assay (Ex 380 nm)

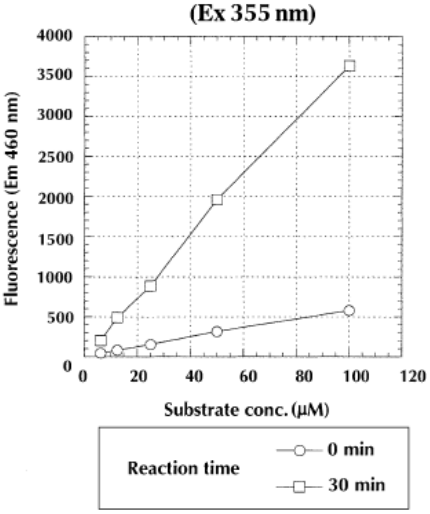


Results

a) Initial rate assay (Ex 390 nm)



b2) End-point assay (Ex 355 nm)



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