Assay Methods Using Peptidyl-AMC (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-AMC), in DMSO at 10 mM
2. AMC stock solution: Content of vial (Code 3099-vAMC), 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

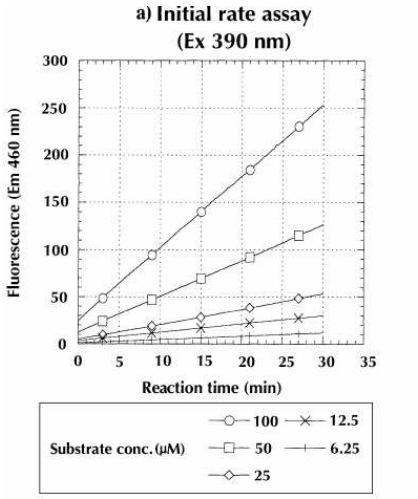
Procedure

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 spectropotometer at λex = 380 nm (or 380 nm, 355 nm) and 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 (x 10, x 20, x 40, x 80, x 160)
3. Caspase-7: Dissolve in buffer
4. Pipette 160 µl of buffer and 20 Ml of substrate solutions (1, ½, ¼, 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 in each well
7. Mount the plate in the fluorescence spectrometer

8 Calculate the amount of released AMC



Results