PROTOCOL

Caspase-6 (Mch2) Assay
Catalog #BML-SE170

Assay buffer
(50 mM HEPES, pH 7.4, 100 mM NaCl, 0.1% CHAPS, 1 mM EDTA, 10% glycerol, 10 mM DTT)

Caspase-6 (BML-SE170)
Dilute to 10 U/µl in assay buffer just before use.

Ac-VEID-pNA (BML-P437) colorimetric substrate
(400 µM stock solution in Assay buffer; Store at -20°C. Warm to assay temperature before use.)
To 5 mg net peptide (MW=637.5) add 784 µl Assay buffer (do not use DMSO), to prepare 10 mM stock. Dilute 10 mM stock to 400 µM with Assay Buffer.

Reaction Conditions :

1) Add 45 µl Assay buffer into 1/2 volume microtiter plate. Allow to equilibrate to assay temperature.

2) Add 5 µl of caspase-6 (10 U/µl) to each appropriate well. Include 2 blanks (5 µl assay buffer rather than caspase-6).

3) To start reaction, add 50 µl Ac-VEID-pNA substrate (400 µM in assay buffer) and mix well by pipetting up and down several times. Final substrate concentration=200 µM.

4) Continuously monitor A_{405nm}.

5) Data analysis: Graph OD vs time and determine slope over the linear portion of the curve. Convert rates in OD/min to substrate/min using an extinction coefficient for p-nitroaniline of 10,500 M^{-1} cm^{-1}, and adjusting for pathlength of sample (~0.5 cm for 100 µl in well of a ½ volume, 96-well plate).