Caspase-3 Colorimetric Substrate, DEVD-pNA

CATALOG NO:  1008-200  200 assays (1 x 1ml vials)
               1008-1000  1000 assays (5 x 1 ml vials)

STORAGE CONDITIONS:  Store at –20 °C, protected from light.

SHELF LIFE:  6 months under proper storage conditions

MOLECULAR WEIGHT:  638.58

SEQUENCE:  Ac-Asp-Glu-Val-Asp-pNA

PURITY:  >98% by HPLC analysis

DESCRIPTION:
Ready-to-use colorimetric substrate for CPP32/caspase-3 and related caspases that recognize
the amino acid sequence DEVD. The sequence DEVD is based on caspase-3 cleavage site in poly
(ADP-ribose) polymerase (PARP). CPP32 and related caspase activity can be quantified
by spectrophotometric detection of free pNA (λ = 400 nm) after cleavage from the peptide
substrate DEVD-pNA, using a spectrophotometer or multi-well plate reader. The ready-to-use
caspase substrate provides an economic alternative for researchers who perform large
amounts of caspase assays.

ASSAY PROCEDURE:
1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture
   without induction.
2. Count cells and pellet 1-5 x 10⁶ cells.
3. Resuspend cells in 50 µl of chilled Cell Lysis Buffer (Cat.# 1067-100) and incubate cells
   on ice for 10 minutes.
4. Centrifuge for 1 min in a microcentrifuge (10,000 x g).
5. Transfer supernatant (cytosolic extract) to a fresh tube and put on ice.
6. Assay protein concentration.
7. Dilute 50-200 µg protein to 50 µl Cell Lysis Buffer for each assay.
8. Add 50 µl of 2X Reaction Buffer (Cat.# 1068-20, -80) containing 10 mM DTT (Cat.# 1201-
   1) to each sample.
9. Add 5 µl of the 4 mM of DEVD-pNA (200 µM final conc.) and incubate at 37 °C for 1-2
   hour.
10. Read samples at 400- or 405-nm in a microtiter plate reader, or spectrophotometer using
    a 100-µl micro quartz cuvet (Sigma), or dilute sample to 1 ml with Dilution Buffer (Cat."
   1066-100, 500) and using regular cuvet (note: Dilution of the samples proportionally
    decreases the reading).

You may also perform the entire assay directly in a 96-well plate.

Fold-increase in caspase activity can be determined by comparing these results with the level
of the uninduced control.

Note: Background reading from cell lysates and buffers should be subtracted from the readings
of both induced and the uninduced samples before calculating fold increase in caspase activity.

FOR RESEARCH USE ONLY! Not to be used in human.