



Caspase-8 Activity Assay Kit

Catalog number: AR4007

This package insert must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.



Catalog Number: AR4007, Storage:

Introduction

Caspase-8, also known as FLICE, MACH, and Mch5, is an intracellular cysteine protease that exists as a proenzyme, becoming activated during the cascade of events associated with apoptosis. Sequential activation of caspases plays a central role in the execution-phase of cell apoptosis. Eight different isoforms of caspase-8 have been reported, each with different functions. Activation of caspase-8 releases two active subunits, p18 and p10, into the cytosol which activate additional caspases that cleave other apoptosis-related substrates such as poly ADP-ribose polymerase (PARP), the 70 kDa protein of the U1-ribonucleoprotein and a subunit of the DNA dependent protein kinase (Casciola-Rosen, L. et al).

Our Caspase-8 Assay Kit, Colorimetric provides a simple and convenient method for assaying the activity of Caspase-8, which is based on the hydrolysis of the labeled substrate Ac-IETD-pNA (N-Acetyl-Ile-Glu-Thr-Asp-p-nitroanilide) by Caspase-8, releasing the pNA (p-nitroaniline) moiety from the substrate. The released pNA has the max absorbance at 405 nm (epsilonmM = 10.5) and its concentration is calculated by measuring the absorbance values at 405 nm or from a standard calibration curve prepared using the pNA Dye Standard, using a microplate reader or spectrophotometer.

The assay can be performed in $100 \,\mu$ l volume in a 96 well plate using an ELISA plate reader or in 1 ml volume and measured in a spectrophotometer, using quartz cuvettes, since plastic cuvettes attenuate the absorption at 405 nm. Comparison of the absorbance of the released pNA from an apoptotic sample with an uninduced control allows determination of the fold increase in Caspase-8 activity.

Overview

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Caspase-8 Activity Assay Kit (Boster Biological Technology, Pleasanton CA, USA, Catalog # AR4007)

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