PRODUCT DATA SHEET

Product: Ac-VAD-pNA (Chromogenic caspase-1, 3, 4, 7 substrate)

Cat. No.: AC-064 (25 mg)

Chemical Name:
Acetyl-Val-Ala-Asp-p-Nitro-Anilide

Molecular Weight: 465

Description:
Lyophilized solid. Chromogenic paranitroanilide substrate of caspase-1. Also cleaved by caspases-3, -4, and -7. Similar to amino acids 114-116 (VHD) of the IL-1B precursor, an endogenous substrate of caspase-1. Release of pNA is monitored at 405 nm (ε=9.160$M^{-1}cm^{-1}$).

Introduction:
Interleukin-1ß Converting Enzyme (ICE), now termed Caspase-1, is a cytoplasmic cysteine protease that cleaves inactive 31 kDa pro-IL-1ß to generate the active 17.5 kDa proinflammatory cytokine IL-1ß, the predominant form of IL-1 produced by human monocytes. This cytokine has been implicated in the pathogenesis of several diseases such as rheumatoid arthritis, inflammatory bowel disease, and septic shock.

Caspase-1/ICE mRNA is found in a variety of cells such as peripheral blood monocytes, peripheral blood lymphocytes, peripheral blood neutrophils, and resting and activated peripheral blood T lymphocytes. The tissue distribution of Caspase-1/ICE suggests that the enzyme may have other substrates in addition to IL-1ß.

Current hypotheses suggest that Caspase-1/ICE is able to cause apoptosis as well as activate inflammation in animal cells. Experiments have shown that Caspase-1/ICE has sequence homology with other mammalian apoptosis genes and that activation of Caspase-1/ICE or other ICE-related proteases (caspases)is required for anti-Fas mAb-induced apoptosis. The role of Caspase-4 in apoptosis is unclear but its substrate specificity is similar to that of Caspase-1/ICE.

Specificity:
Substrate for caspase-1. Also cleaved by caspases-3, -4, and -7.

Applications:
Assay of caspase activity in cell extracts.

Protocol:
Soluble in DMSO and aqueous buffers. We recommend preparing a stock solution in high purity DMSO (>99.9%), and diluting into aqueous buffer shortly prior to use.

Suggested procedure only. Each laboratory must determine its own conditions.

1. Lyse cells in 50 mM Tris-HCl, pH 7.5, 0.3% NP-40, 1.0 mM DTT, at a density of 2 X 10^6/ml.

2. Assay 0.01 ml cell lysate in a final volume 0f 0.1 ml. Assay buffer is cell lysis buffer containing 0.2 mM substrate.

3. Incubate at 37C for 0-3 hr. Take periodic readings of absorbance at 405 nm.

Storage and Stability:
Solid can be stored at room temperature. Protect from light and moisture. Store stock solutions in DMSO refrigerated or frozen. Stable indefinitely protected from light and moisture. Stock solutions in DMSO can be stored for long periods refrigerated or frozen.. Solutions in aqueous buffers should be stored for only short periods of time. Hydrolysis of the substrate will be revealed by the appearance of a yellow color.

Limitations:
For *in vitro* research use only. Not for use in diagnostics or in humans.

Warranty:
No warranties, expressed or implied, are made regarding the use of this product. KAMIYA BIOMEDICAL COMPANY is not liable for any damage, personal injury, or economic loss caused by this product.