



## PRODUCT DATA SHEET

**Product:** Ac-VEID-pNA (Chromogenic caspase-3, 6 substrate)

**Cat. No.:** AC-026 (25 mg)

**Chemical Name:**

Acetyl-Val-Glu-Ile-Asp-pNA

**Formula:**

C<sub>28</sub>H<sub>40</sub>N<sub>6</sub>O<sub>11</sub>

**Molecular Weight:**

636

**Purity:**

>98% by HPLC

**Description:**

Chromogenic paranitroanilide-peptide substrate for caspase-6 (Mch2). Release of free pNA is monitored by absorbance at 405 nm ( $\epsilon=9,160 \text{ M}^{-1}\text{cm}^{-1}$ ).

**Form:**

White lyophilized solid.

**Introduction:**

Caspase-6 (also known as Mch2) is a member of the caspase family of cysteine proteases involved in apoptosis. It is a member of the Group III caspases (6, 8, and 9) which prefer the (L/V) EXD sequence as a substrate. Caspase-6 prefers a hydrophobic amino acid at P4, along with caspases-1 and -4, as opposed to the preference for Asp seen with caspases-2, -3, and -7. This is at odds with the gene sequence alignment that predicts Caspase-6 is more closely related to caspases-3 and -7 than to caspase-1. The preference by Caspase-6 for  $\beta$ -branched amino acids in P4 fits well with the one known natural substrate, lamin A, and distinguishes it from caspases-1 and -4. Reconstitution experiments indicate that Caspase-6 activates caspases-3 and -7 and is therefore part of the proteolytic cascade that initiates apoptosis.

**Specificity:**

Substrate for caspase-3, 6.

**Applications:**

Assay of caspase activity in cell extracts.

**Protocol:**

Soluble in DMSO and aqueous buffers. We recommend preparing a stock solution in DMSO, and diluting into aqueous buffer shortly prior to use.

Suggested procedure only. Each laboratory must determine optimum conditions.

1. Lyse cells in 50 mM Tris-HCl, pH 7.5, 0.3% NP-40, 1.0 mM DTT, at a density of  $2 \times 10^6$ /mL.
2. Assay 0.01 mL cell lysate in a final volume of 0.1 mL. Assay buffer is cell lysis buffer containing 0.2 mM substrate.
3. Incubate at 37°C for 0-3 hr. Take periodic readings of absorbance at 405 nm.

**Storage and Stability:**

Solid can be stored at room temperature. However, for long term storage, desiccated at 4°C is recommended. 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.

**Reference:**

1. Thornberry, N. et al. (1997). *J. Biol. Chem.* **272**(29): 17907-17911.

**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.