



## PRODUCT DATA SHEET

**Product:** Ac-IETD-pNA

**Cat. No.:** AC-025 (25 mg)

**Chemical Name:**

Acetyl-Ile-Glu-Thr-Asp-pNA

**Formula:**

$C_{27}H_{38}N_6O_{12}$

**Molecular Weight:**

638

**Purity:**

98 ± 1% by HPLC.

**Description:**

Lyophilized solid. Chromogenic paranitroanilide-peptide substrate for caspase-8. Also cleaved by granzyme B. Release of free pNA is monitored by absorbance at 405 nm ( $\epsilon=9,160 \text{ M}^{-1}\text{cm}^{-1}$ ).

**Introduction:**

Caspase-8/FLICE (also known as MACH or Mch5) is a member of the caspase family of cysteine proteases. Caspases play an important role in apoptosis signaling and effector mechanisms. Caspase-8 is most similar to caspase-10, both of which have "death domain" motifs. Caspase-8 appears to be physically associated with the signaling mechanism during Fas-mediated cell death and its association with Fas or tumor necrosis factor receptors via an interaction with FADD suggests it functions as an initiator rather than an effector of the cell death pathway. Thus, Caspase-8 is an upstream activator in the protease cascade that proteolytically matures other caspases.

**Specificity:**

Substrate for caspase-8. Also cleaved by granzyme B.

**Applications:**

Assay of caspase activity in cell extracts.

**Protocol:**

Soluble in DMSO and aqueous buffers. We recommend preparing a stock solution in high purity (>99.9%) 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.

**Form:**

White lyophilized powder.

**Storage and Stability:**

Solid can be stored desiccated at room temperature. Protect from light and moisture. For long term storage, desiccated at 4°C is recommended. Store stock solutions in DMSO refrigerated or frozen. 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.