



## PRODUCT DATA SHEET

**Product:** Ac-IEPD-pNA (Chromogenic Granzyme B Substrate)

**Cat. No.:** AC-030 (25 mg)

**Chemical Name:**

Acetyl-Ile-Glu-Pro-Asp-paranitroanilide

**Molecular Weight:**

634

**Purity:**

>98% by HPLC

**Formula:**

$C_{28}H_{38}N_6O_{11}$

**Description:**

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

**Inhibitory data:**

Steady-state kinetic parameters for the hydrolysis of Ac-IEPD-pNA by recombinant granzyme B:

$$K_{cat} = 4.16 \text{ S}^{-1}$$

$$K_m \times 10^{-6} = 57 \text{ M}$$

$$K_{cat}/K_m = 66,600 \text{ S}^{-1}\text{M}^{-1}$$

**Specificity:**

Chromogenic substrate for granzyme B.

**Form:**

White solid

**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  $2 \times 10^6/\text{mL}$ .
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 hrs. Take periodic readings of absorbance at 405 nm.

**Storage:**

Ac-IEPD-pNA can be stored desiccated at room temperature. Protect from light and moisture. Suggested long term storage is desiccated at 4°C. DMSO stock solutions have a shelf-life of 6-8 months at -20°C if care is taken by choosing DMSO with maximum dryness (>99.9%). Keep sealed after removing from the freezer until the temperature of the vial equilibrates with room temperature. 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.