

PRODUCT DATA SHEET

Product: Ac-DEVD-pNA

Cat. No.: AC-024 (25 mg)

Chemical Name:

Acetyl-Asp-Glu-Val-Asp-pNA

Formula:

 $C_{26}H_{34}N_6O_{13}$

Molecular Weight: 638

Form: White lyophilized powder

Purity:

>97% by HPLC

Description:

Chromogenic paranitroanilide-peptide substrate for caspase-3 (Km = 9.7 μ M). Ac-DEVD-pNA is based on amino acids 213-216 in poly (ADP-ribose) polymerase (PARP), an endogenous substrate for caspase-3. This substrate is also cleaved by caspases-6, -7, -8, and -10. Release of free pNA is monitored by absorbance at 405 nm (ϵ = 9,160 M⁻¹cm⁻¹).

Introduction:

Caspase-3/CPP32 is a member of the cysteine proteases family involved in apoptosis induction. All apoptotic pathways studied to date involve proteolytic activation of Caspase-3/CPP32 as a central event in the progression of cell death. Although the death-inducing consequences of Caspase-3/CPP32 activation have not been conclusively established, several crucial substrates for the protease have been identified in vitro, including DNA-dependent protein kinase, Poly (ADP-ribose) Polymerase (PARP), Replication factor C, and Gelsolin. These substrates are involved in the later stages of apoptosis, strongly suggesting that Caspase-3/CPP32 has a key role in promoting the final processes leading to cell death.

Specificity:

A substrate for caspase-3 and related cysteine proteases. Also cleaved by caspase-6, caspase-7, caspase-8 and caspase-10.

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 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 x 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:

Store Ac-DEVD-pNA in a desiccator 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. Stable indefinitely when protected from light and moisture. 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.