Product Data Sheet

Product Name:	Caspase 3 (Apopain) Substrate 1, chromogenic
Catalog Number:	AS-25263-5 (5 mg) Lot Number: See label on vial
Sequence:	Ac-Asp-Glu-Val-Asp-pNA (3-letter code) Ac-DEVD-pNA (1-letter code)
Molecular Weight:	638.6
% Peak Area by HPLC:	≥ 95
Appearance:	Lyophilized white powder

Peptide Reconstitution: Use fresh concentrated Anhydrous DMSO as the solvent. Do not use DMSO diluted with aqueous solvents, as this is not an effective solvent. Do not add aqueous solvent to the peptide prior to DMSO addition as this may prevent proper solubilization. Add concentrated DMSO directly to the lyophilized peptide powder to obtain a final concentration of approximately 0.5mg/mL to 1mg/mL. Gently vortex to mix.

Storage: Peptide is shipped at ambient temperature. Upon receipt, store lyophilized powder at –20°C or lower. Reconstituted peptide should be aliquoted into several freezer vials and stored at –20°C or lower. Do not freeze thaw.

Description: pNA (4-nitroaniline)-derived caspase substrates are widely used for the colorimetric detection of various caspase activities. Cleavage of pNA peptides by caspases generates pNA that is monitored colorimetrically at ~405 nm. pNA has maximum absorption around 408 nm.

Caspase-1 substrate with Km = 18 uM and kcat = 0.5 M-1s-1; Caspase-3 substrate with Km = 11 uM and kcat = 2.4 M-1s-1;Caspase-4 substrate with Km = 32 uM and kcat = 0.05 M-1s-1 Caspase-6 substrate with Km = 180 uM and kcat = 0.6 M-1s-1 ; Caspase-7 substrate with Km = 12 uM and kcat = 0.4 M-1s-1; Caspase-8 substrate with Km = 167 uM

Ref: Gastman, BR. *Head Neck* **23**, 409 (2001); Grutter, MG. *Curr Opin Struct Biol* **10**, 649 (2000); Koeplinger, KA. et al. *Protein Expr Purif* **18**, 378 (2000); Stennicke, HR. et al. *Cell Death Differ* **6**, 1054 (1999); Fassy, F. et al. *Eur J Biochem* **253**, 76 (1998); Stennicke, HR. et al. *Biochim Biophys Acta* **1387**, 17 (1998); Thornberry, NA. et al. *Sci* **281**, 1312 (1998); Talanian, RV. et al. *J Biol Chem* **272**, 9677 (1997); Datta, R. et al. *Blood* **88**, 1936 (1996)

Additional Information: *Listed below are relevant information that may provide a guideline on how to use this product. End users will have to adapt to their own specific applications.*

Ac-DEVD-pNA (caspase-3 substrate) and Ac-LEHD-pNA (caspase-9 substrate) were procured from Anaspec. Caspase assay were performed by pipetting 40 μl cell lysates to a 96-well dish, containing 158 μl reaction buffer (20% glycerol; 0.5 mM EDTA; and 5 mM DTT; 100 mM Hepes, pH 7.5), and 2 μl fluorogenic Ac-DEVD-pNA or Ac-LEHD-pNA. Samples were incubated for 8 h at 37 °C and determined at 405 nm in an ELISA reader (Molecular Devices)- Yiang, GT. et al. *J FEBS Lett* **582**, 881 (2008).

Ac-LEHD-pNA (acetyl–Leu–Glu–His–Asp–*p*-nitroanilide), Ac-DEVD-pNA (acetyl-Asp–Glu–Val–Asp–*p*-nitroanilide), and Ac-IETD-pNA (acetyl-Ile–Glu–Thr–Asp–*p*-nitroanilide) were purchased from Anaspec. Cells were resuspended in the lysis buffer (50 mM Tris–HCl, 120 mM NaCl, 1 mM EDTA, and 1% NP-40, pH 7.5) supplemented with protease inhibitors. Insoluble pellets were removed by centrifugation using 15 000 × *g* at 4 °C for 20 min. The caspase activity

assay was performed in a reaction containing 40 μl cell lysates (80 μg total protein), 158 μlreaction buffer (20% glycerol, 0.5 mM EDTA, 5 mM dithiothreitol, and 100 mM HEPES, pH 7.5), and 2 μl fluorogenic Ac-LEHD-pNA, Ac-DEVD-pNA, or Ac-IETD-pNA substrates (100 μM final concentration); the reaction was incubated at 37 °C for 6 h (in this condition, all substrates were not used up and the caspase activity could be compared in the linear range)- Tang, C-HA. et al. *J FEBS Lett* **579**, 265 (2005).

Acetyl-Asp-Glu-Val-Asp-*p*-nitroanilide (Ac-DEVD-pNA) and acetyl-Leu-Glu-His-Asp-*p*nitroanilide (Ac-LEHD-pNA) were procured from Anaspec. Cells were resuspended in lysis buffer (50 mM Tris–HCl, 120 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, pH 7.5) supplemented with protease inhibitors. Cell lysates were obtained after centrifugation (15 000×*g*) for 20 min at 4°C. Caspase activity assays were performed as previously described. In brief, the whole reaction contained 40 μl cell lysates (80 μg total protein), 158 μl reaction buffer (20% glycerol, 0.5 mM EDTA, 5 mM dithiothreitol, 100 mM HEPES, pH 7.5), and 2 μl fluorogenic Ac-DEVD-pNA or Ac-LEHD-pNA substrates (100 μM final concentration). Samples were incubated for 6 h at 37°C and enzyme-catalyzed release of *p*-nitroanilide was monitored at 405 nm in an ultra-microplate reader (Bio-Tek instruments)- Wei, C-W. et al. *J FEBS Lett* **531**, 421 (2002).

Published Citations:

Kang,, HJ. et al. *BMC Biotech* **8**, 92 (2008).
Yiang, GT. et al. *J FEBS Lett* **582**, 881 (2008).
Tang, C-HA. et al. *J FEBS Lett* **579**, 265 (2005).
Wei, C-W. et al. *J FEBS Lett* **531**, 421 (2002).

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