



Product Data Sheet

Product Name:	Caspase 9 Substrate 1, chromogenic	
Catalog Number:	AS-25278-5 (5 mg)	Lot Number: See label on vial
Sequence:	Ac-LEHD-pNA (1-letter code)	
Molecular Weight:	675.7	
% Peak Area by HPLC:	≥ 95	
Appearance:	Lyophilized off-white powder	

Peptide Reconstitution: Reconstitute by adding 50-70 μ l 1% NH_4OH to 1 mg Caspase 9 peptide. Dilute this peptide solution to approximately 1 mg/ml (or more dilute) with a buffer such as PBS or another buffer; aliquot and store at -20°C .

Storage: Caspase 9 peptide is shipped at ambient temperature. Upon receipt, store lyophilized peptide at -20°C or lower. Reconstituted peptide can be aliquoted and stored at -20°C or lower.

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

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.

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. In brief, the whole reaction contained 40 μ l cell lysates (80 μ g total protein), 158 μ l reaction buffer (20% glycerol, 0.5 mM EDTA, 5 mM dithiothreitol, 100 mM HEPES, pH 7.5), and 2 μ l fluorogenic Ac-DEVD-pNA or Ac-LEHD-pNA substrates (100 μ M 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 Letters. 531, 421 \(2002\).](#)

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. The caspase activity assay was performed in a reaction containing 40 μ l cell lysates (80 μ g total protein), 158 μ l reaction buffer (20% glycerol, 0.5 mM EDTA, 5 mM dithiothreitol, and 100 mM HEPES, pH 7.5), and 2 μ l fluorogenic Ac-LEHD-pNA, Ac-DEVD-pNA, or Ac-IETD-pNA substrates (100 μ M 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). The fluorogenic substrate cleavage readout was the p-nitroanilide release as detected at 405 nm in an ultra-microplate reader (Bio-Tek instruments)- [Tang, C-HA. et al. J. FEBS Letters. 579, 265 \(2005\).](#)

Ac-DEVD-pNA (caspase-3 substrate) and Ac-LEHD-pNA (caspase-9 substrate) were procured from Anaspec. Cell lysates were obtained by treating cells with a lysis buffer (50 mM Tris-HCl; 120 mM NaCl; 1 mM EDTA; 1% NP-40, pH 7.5) supplemented with protease inhibitors (Calbiochem). 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-[Yiang, GT. et al. FEBS Letters 582, 881 \(2008\).](#)

Published Citations:

Wei, C-W. et al. *J. FEBS Letters*. **531**, 421 (2002).
Tang, C-HA. et al. *J. FEBS Letters*. **579**, 265 (2005).
Yiang, GT. et al. *FEBS Letters* **582**, 881 (2008).

Related Products:

Name	Cat #	Size
Caspase 9 Substrate 1f, fluorogenic, Abs/Em=370/500 nm (Ac-LEHD-AFC)	AS-25276-5	5 mg
Caspase 9 Substrate 2m, fluorogenic, Abs/Em=340/440 nm (Ac-LEHD-AMC)	AS-25286-5	5 mg

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