



PRODUCT DATA SHEET

Product: Ac-LEHD-pNA (Chromogenic caspase-4, 5, 9 substrate)

Cat. No: AC-028 (25 mg)

Chemical Name:

Acetyl-Leu-Glu-His-Asp-pNA.TFA

Molecular Weight:

788 (with TFA salt)

674 (without TFA salt)

Purity:

>97% by HPLC

Description:

Lyophilized solid. TFA salt of a paranitroanilide-peptide substrate for caspases-4, -5, and -9. Release of free pNA is monitored by absorbance at 405 nm ($\epsilon=9,160 \text{ M}^{-1}\text{cm}^{-1}$).

Introduction:

Caspase-4 (also known as ICERel-II, TX, or ICH-2), Caspase-5 (also known as ICERel-III or TY), and Caspase-9 (also known as ICE-LAP6 or Mch6) are members of the caspase family of cysteine proteases involved in apoptosis. Caspases-4 and -5 belong to Group I (along with caspase-1), which prefer the tetrapeptide substrate sequence WEHD and are thought to be involved in inflammation through the maturation of pro-IL-1 β . Their role in apoptosis, however, is unclear. Caspase-9 is a member of Group III, which prefer the substrate sequence (L/V)_jEXD. Since Caspase-9 has a strict requirement for His in the P4 position, it is not unexpected that the LEHD inhibitor sequence would work well on this caspase. The Group III caspases optimal recognition sequence resembles the activation sites within several effector caspase proenzymes, implicating the Group III enzymes as upstream components in the proteolytic cascade that amplifies the death signal.

Specificity:

Serves equally well as a substrate for Caspases-4, -5, and -9. Can also serve as a weak substrate for Caspases-1, -2, -6, and -8 at an efficiency of 30-40% .

Applications:

For in vitro assays of Caspases-4, -5, and -9 activity. Can be used with purified or partially purified enzymes, or possibly with crude cell

lysates (if the Caspase-4,5,9 Inhibitor is included to determine background protease activity).

Protocol:

Soluble in DMSO and aqueous buffers. We recommend preparing a stock solution in 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 2×10^6 /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:

Solid can be stored at room temperature. Protect from light and moisture. Store stock solutions in DMSO refrigerated or frozen. Stable indefinitely protected from light and moisture. 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.

References:

1. Duan, H. et al. (1996). *J. Biol. Chem.* 271: 16720-24.
2. Faucheu, C. et al. (1995). *EMBO J.* 14: 1914-22.
3. Kamens, J. et al. (1995). *J. Biol. Chem.* 270: 15250-56.
4. Munday, N.A. et al. (1995). *J. Biol. Chem.* 270: 15870-76.
5. Srinivasula, S.M. et al. (1996). *J. Biol. Chem.* 271: 27099-106.
6. Talanian, R.V. et al. (1997). *J. Biol. Chem.* 272: 9677-82.
7. Thornberry, N. et al. (1997). *J. Biol. Chem.* 272(29): 17907-911.

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.