



PRODUCT DATA SHEET

Product: Ac-WEHD-pNA (Chromogenic caspase-1, 4, 5 substrate)

Cat. No: AC-029 (25 mg)

Chemical Name:

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

Formula:

C₃₄H₃₇N₉O₁₁

Molecular Weight: 747

Purity:

>97% by HPLC

Description:

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

Introduction:

Caspase-1 (also known as ICE), Caspase-4 (also known as ICERel-II, TX, or ICH-2), and Caspase-5 (also known as ICERel-III or TY) make up the Group I caspases, all of which prefer the tetrapeptide substrate sequence WEHD. Although Group I caspases are involved in inflammation through the maturation of pro-IL-1 β There is evidence suggesting that activation of Group I caspases induces apoptosis, although substrate specificity studies do not provide compelling evidence for a role of Group I caspases in apoptosis since hydrophobic amino acids in the P4 position (preferred by Group I) are not observed in proteins cleaved during apoptosis. Most research supports a role for Caspase-1 in inflammation but the roles of Caspase-4 and Caspase-5 have not been established.

Specificity:

Substrate for caspase-1, caspase-4, and caspase-5.

Applications:

Assay of caspase activity in cell extracts.

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.

Reference:

1. Faucheu, C. et al. (1995). *EMBO J.* 14: 1914-22.
2. Kamens, J. et al. (1995). *J. Biol. Chem.* 270: 15250-56.
3. Munday, N.A. et al. (1995). *J. Biol. Chem.* 270: 15870-76.
4. Talanian, R.V. et al. (1997). *J. Biol. Chem.* 272: 9677-82.
5. 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.