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ProductInformation

CAPASE 6, HUMAN RECOMBINANT
C-terminal histidine tagged protein
Expressed in *E. coli*

Product Code **C 4977**
Storage Temperature –70 °C

Synonyms: Mch2

Product Description
Caspase-6 proenzyme is expressed in *E. coli* as a C-terminal histidine-tagged protein. It undergoes autoactivation and autoprocessing to 21 and 19 kDa protein polypeptides (large subunit), and 14 kDa polypeptide (small subunit).[1,2]

Caspase-6 (Mch2) is a member of the CED-3 subfamily of the caspase family of cysteine proteases that play an essential role in the execution phase of apoptosis. These enzymes share a dominant primary specificity for cleaving bonds following aspartic acid residues. "Initiator" caspases (such as caspase-8) activate "effector" caspases (e.g. caspase-3 and –7). The effector caspases then cleave cellular substrates ultimately leading to the morphological changes of apoptosis.[3-5] Caspase-6 is an effector caspase, localized downstream of caspase-3.[6] It has high homology with caspase-3.[4]

Caspases are synthesized as inactive proenzymes. The precursor proteins contain N-terminal pro-sequences of various lengths followed by the p20 and p10 subunits. Caspases are activated by cleavage at specific Asp residues to produce two subunits of approximately 20 kDa (p20) and 10 kDa (p10), which together form the heterodimeric active protease.[4,5] In some cases, these subunits are separated by a linker that may be involved in regulation of the activation of the caspase. All caspases contain an active-site pentapeptide of general structure QACXG (where X is R, Q or G). The amino acids Cys-285 and His-237 involved in catalysis, and those involved in forming the P1 carboxylate binding pocket (Arg-179, Gln-283, Arg-341 and Ser-347) are conserved in all caspases, except for the substitution of Thr for Ser-347 in caspase-8. This explains the absolute requirement for an Asp in the P1 position. Residues forming the P2–P4 binding pocket are not well conserved. This suggests they may determine the substrate specificities of the caspases. Evidence suggests that not all caspases are required for cell death, and some caspases appear to be more important than others.[4]

Procaspase-6 is can be cleaved by caspases-3, -8, -9 and granzyme B.[7-11] Once activated, caspase-6 cleaves lamin A to its signature apoptotic fragment indicating that caspase-6 is an apoptotic laminase.[11-12] It also cleaves PARP[11], NuMa (nuclear mitotic apparatus protein)[13], and keratin 18.[14] Furthermore, the cytochrome c induced processing of caspases 8 and 10 may depend on caspase-6.[15] Caspase-6 is inhibited by VEID-CHO and CrmA (cytokine response modifier A).[13]

Reagent
The product is supplied as a solution in 10% sucrose containing 50 mM HEPES pH 7.5, 0.5 M NaCl, 100 mM imidazole, 0.1% CHAPS, and 4 mM DTT.

Storage/Stability
Store at –70 °C. Aliquot to avoid repeated freeze and thaw cycles. Storage in “frost-free freezers is not recommended.

Assay Procedure
The assay is based on the hydrolysis of the peptide substrate Acetyl-Val-Glu-Ile-Asp-p-Nitroaniline (Ac-VEID-pNA) by caspase-6 resulting in the release of a p-Nitroaniline (pNA) moiety. p-Nitroaniline has high absorbance at 405 nm (ε_{mM} = 10.5). The concentration of the pNA released from the substrate is calculated from the absorbance values at 405nm.

Ac-VEID-pNA ⟶ pNA (OD 405 nm)

Reagents
Note: Use 17 megohm water for buffer preparation

Assay buffer: 20 mM HEPES, pH 7.4,2 mM EDTA, 0.1% CHAPS, 5 mM DTT, 5% sucrose.

Substrate (Ac-VEID-pNA, Product Code A 5220) stock solution (20 mM in DMSO): add 0.39 ml DMSO to 5 mg of Ac-VEID-pNA (MW 636.7). Store at –20 °C.

Substrate working solution (2 mM Ac-VEID-pNA): Dilute the Ac-VEID-pNA stock solution 10 fold in assay buffer. Prepare 50 µl per 0.5 ml reaction.

Equipment
0.5 ml quartz cuvette
Thermoregulated spectrophotometer (25 °C)

Reaction Scheme

	Assay Buffer	Caspase-6 0.1 - 0.2 mg/ml	Ac-VEID-pNA Working solution
Blank	450 µl	---	50 µl
Test	445-447 µl	2.5 – 5 µl	50 µl

Note: perform the assay in duplicates.

1. Pre-warm assay buffer to 25 °C.
2. Add assay buffer into polypropylene tubes.
3. Add Caspase-6 solution to the appropriate tubes
4. Start the reaction by adding 50 µl of substrate working solution.
5. Incubate for 5 min at 25 °C.
6. Determine OD of sample against blank.

Calculations:

$$Unit/ml = \frac{OD}{e_{mM}} \times \frac{1}{t} \times \frac{v}{V} = \frac{OD}{0.0105} \times \frac{1}{5} \times \frac{0.5}{V}$$

Activity: Units/ml
Unit definition: One unit is the amount of enzyme that will cleave 1.0 nmol of the substrate Ac-VEID-pNA per minute at 25 °C, pH 7.4
$OD = OD_{sample} - OD_{blank}$
$\varepsilon_{\mu M} = 0.0105$
V = Volume of diluted enzyme in the reaction in ml (0.0025-0.005 ml)
v = reaction volume in ml
t = reaction time in min (5 min)

Product Profile
Purity: >90% (SDS PAGE)
Activity > 1000 units per mg protein

References

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Related Products
Substrates: N-Acetyl-Val-Glu-Ile-Asp 7-Amido-4-trifluoromethylcoumarin (Ac-VEID-AFC), Product Code A 5095;
N-Acetyl-Val-Glu-Ile-Asp p-nitroanilide (Ac-VEID-pNA), Product Code A 5220
Inhibitors: N-Acetyl-Val-Glu-Ile-Asp-al (Ac-VEID-CHO), Product Code A 6339;
N-CBZ-Val-Glu-(OME)-Ile-Asp(OME) fluoromethyl ketone (Z-VEID-FMK), Product Code C 1730