DESCRIPTION

Source
E. coli-derived
Ser29-Asp175 (subunit 1) & Ala183-His277 (Asp190Glu) (subunit 2)
Accession # P42574

N-terminal Sequence Analysis
Ser29 (subunit 1) & Ala183 (subunit 2)

Predicted Molecular Mass
17 kDa (subunit 1), 11 kDa (subunit 2)

SPECIFICATIONS

SDS-PAGE
18 kDa and 10 kDa, reducing conditions

Activity
Measured by its ability to cleave the fluorogenic peptide substrate Ac-DEVD-AFC. The specific activity is >3,000 pmol/min/μg, as measured under the described conditions.

Endotoxin Level
<1.0 EU per 1 μg of the protein by the LAL method.

Purity
>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

Formulation
Supplied as a 0.2 μm filtered solution in HEPES, NaCl, DTT and Sucrose with BSA as a carrier protein. See Certificate of Analysis for details.

Activity Assay Protocol

Materials
- Assay Buffer: 25 mM HEPES, 0.1% (w/v) CHAPS, 10 mM dithiothreitol (DTT), pH 7.5
- Recombinant Human Caspase-3 (rhCaspase-3) (Catalog # 707-C3)
- Substrate: Ac-Asp-Glu-Val-Asp-AFC (MP Biomedicals, Catalog # AFC138), 10 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay
1. Dilute rhCaspase-3 to 0.4 ng/μL in Assay Buffer.
2. Dilute Substrate to 100 μM in Assay Buffer.
3. Load 50 μL of 0.4 ng/μL rhCaspase-3 into a plate, and start the reaction by adding 50 μL of 100 μM Substrate. Include a Substrate Blank containing 50 μL Assay Buffer and 50 μL of 100 μM Substrate.
4. Read at excitation and emission wavelengths of 400 nm and 505 nm (top read), respectively, in kinetic mode for 5 minutes.
5. Calculate specific activity:
   Specific Activity (pmol/min/μg) = \frac{Adjusted V_{max}^* \times (RFU/min) \times Conversion Factor**}{amount of enzyme (μg)}
   *Adjusted for Substrate Blank
   **Derived using calibration standard 7-amino, 4-(trifluoromethyl)coumarin (Calbiochem, Catalog #164580).

Final Assay Conditions
- Per Well:
  - rhCaspase-3: 0.02 μg
  - Substrate: 50 μM

PREPARATION AND STORAGE

Shipping
The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.

Stability & Storage
Use a manual defrost freezer and avoid repeated freeze-thaw cycles.
- 6 months from date of receipt, -70 °C as supplied.
- 3 months, -70 °C under sterile conditions after opening.
Caspase-3 (Cysteine-aspartic acid protease 3/Casp3; also Yama, apopain and CPP32) is a 29 kDa member of the peptidase C14A family of enzymes (1, 2, 3). It is widely expressed and is an integral component of the apoptotic cascade. Caspase-3 is considered to be the major executioner caspase; that is, the primary downstream mediator of apoptotic-associated proteolysis (2, 3, 4). Active Caspase-3 is known to utilize a Cys residue to cleave multiple substrates, including PARP, proIL-16, PKC-γ & -δ, procaspases 6, 7 and 9, and β-catenin (1). Human procaspase-3 is a 32 kDa, 277 amino acid (aa) protein (5, 6, 7). Normally, it is an inactive, cytosolic homodimer, but following an upstream signal that activates processing proteases, procaspase-3 undergoes proteolytic cleavage (1, 2, 8, 9). This generates an N-terminal 175 aa p20/20 kDa subunit plus a 102 aa C-terminal p12/12 kDa subunit, followed by further processing of the p20 subunit at Asp28 to generate a final p17 subunit (aa 29-175) (9). The p17 and p12 subunits noncovalently heterodimerize, and subsequently associate with another p17/p12 heterodimer to form an active antiparallel homodimer. The p17 subunit contains the enzyme active site (aa 161-165), with an embedded catalytic Cys which is normally nitrosylated and inactive. Full activation requires both proteolytic processing and Cys163 denitrosylation (10). Multiple proteases can use Caspase-3 as a substrate including Caspase-6, -8, and -10, granzyme B, and Caspase-3 itself (9, 11, 12, 13).

References: