Recombinant Human Pro-Caspase-3

Catalog Number: 731-C3

DESCRIPTION

Source	*E. coli*-derived Met1-His277, with an N-terminal Met and 6-His tag Accession # AAA65015
N-terminal Sequence Analysis	Not tested: Met predicted.
Structure / Form	Pro form
Predicted Molecular Mass	33 kDa

SPECIFICATIONS

SDS-PAGE	35 kDa, reducing conditions
Activity	The activity of the activated Caspase-3 was measured by its ability to cleave DEVD-AFC. The specific activity is >500 pmol/min/µg, as measured under the described conditions. See Activity Assay Protocol on www.RnDSystems.com
Purity	>90%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Lyophilized from a 0.2 µm filtered solution in Tris, NaCl, Sucrose and DTT. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 20 mM Tris, 0.3 M NaCl, 0.05% (w/v) CHAPS, 5 mM DTT, 5% (w/v) Sucrose, pH 8.0
- Recombinant Human Pro-Caspase-3 (rhPro-Caspase-3) (Catalog # 731-C3)
- Recombinant Human Caspase−8 (rhCaspase-8) (Catalog # 705-C8)
- 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. Activate rhPro-Caspase-3 at 10 µg/mL with 10 µg/mL rhCaspase-8 at a final concentration, respectively in Assay Buffer.
2. Incubate reaction at 37 °C for 90 minutes.
3. Dilute Substrate to 100 µM in Assay Buffer.
4. Dilute Activated rhPro-Caspase-3 to 0.4 µg/mL.
5. In a plate load 50 µL of 0.4 µg/mL rhPro-Caspase-3 and include a Substrate Blank containing 50 µL Assay Buffer.
6. Start the reaction by adding 50 µL of 100 µM Substrate to wells.
7. Read at excitation and emission wavelengths of 400 nm and 505 nm (top read), respectively in kinetic mode for 5 minutes.
8. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (RFU/min) x Conversion Factor** (pmol/RFU)}}{\text{amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard 7-amino, 4-(trifluoromethyl), Coumarin (Biomedicals, Catalog # 164580).

Final Assay Conditions

Per Well:

- rhPro-Caspase-3: 0.02 µg
- Substrate: 50 µM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 50 µg/mL in 20 mM Tris, 300 mM NaCl, 5 mM Dithiothreitol, 5% Sucrose and 0.05% CHAPS, pH 8.0.
Shipping	The product is shipped at ambient temperature. 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, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after reconstitution.

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BACKGROUND

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).

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