Recombinant Human Caspase-7

Catalog Number: 823-C7/CF

DESCRIPTION

Source	*E. coli*-derived human Caspase-7 protein Ala24-Asp198 (p20) & Ala207-Gln303 (p11) Accession # P55210
N-terminal Sequence Analysis	Ala24 (p20) & Ala207 (p11)
Predicted Molecular Mass	19-20 kDa (p20) & 11 kDa (p11)

SPECIFICATIONS

SDS-PAGE	19-20 kDa and 11 kDa, under reducing conditions.
Activity	Measured by its ability to cleave the fluorogenic peptide substrate Ac-DEVD-AFC. The specific activity is >3300 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 under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 µm filtered solution in HEPES, NaCl, DTT and Sucrose. 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-7 (rhCaspase-7) (Catalog # 823-C7/CF)
- 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-7 to 0.2 ng/µL in Assay Buffer.
2. Dilute Substrate to 100 µM in Assay Buffer.
3. Load 50 µL of 0.2 ng/µL rhCaspase-7 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:

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

*Adjusted for Substrate Blank

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

Final Assay Conditions

Per Well:

- rhCaspase-7: 0.010 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.

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BACKGROUND

Caspase-7 (**C**ysteine-**asp**artic acid prote**ase** 7/Casp7; also CMH-1, ICE-LAP3 and Mch3) is a 32 kDa member of the peptidase C14A/IL-1β-converting family of enzymes (1, 2, 3). It is widely expressed, except in brain, and is best known as an integral component of the apoptotic cascade. Caspase-7 is considered to be an executioner caspase, as a downstream mediator of apoptotic-associated proteolysis (2, 3). Upon activation, Caspase-7 is known to utilize a Cys residue to cleave multiple substrates, including PARP, procaspase 6, Gas2 and calpstatin (1). Human procaspase-7 is a 34-36 kDa, 303 amino acid (aa) protein (4, 5, 6). Normally, it is an inactive homodimer (1, 2, 7, 8). But following an upstream signal that activates processing proteases, procaspase-7 undergoes proteolytic cleavage to generate an N-terminal 23 aa propeptide, a 175 aa p20/20 kDa subunit (aa 24-198), and a 105 aa C-terminal p12/12 kDa subunit (5). The p20 and p12 subunits noncovalently heterodimerize, and subsequently associate with another p20/p12 heterodimer to form an active antiparallel homodimer. Additional processing of p20 may remove aa 24-36 to generate p18, while additional processing of p12 will remove aa 199-206 to generate p11 (9, 10). Multiple proteases can use Caspase-7 as a substrate, and include caspase-1, -3, -8, and -10, granzyme B, calpain-1 and Caspase-7 itself (3, 6, 9, 11). Caspase-7 is found in both cytosol and nucleus, and possesses a potential KKKK nuclear localization signal between aa 38-41 that likely undergoes sumoylation (9, 12). There are two potential isoform variants, one which shows an alternate start site 33 aa upstream of the standard start site, and a second that shows a 105 aa substitution for aa 149-303. Human and mouse Caspase-7 are 82% aa identical at the amino acid level.

References:

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