CAPN9

PDB:1ZIV

Revision

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Entry Clone Accession:BC027993
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Tag:N-terminal histag with integrated thrombin cleavage site: mgsshhhhhhssglvpr*gs
Host:BL21(DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsSFEQMRQECLQRGTLFEDADFPASNSSLFYSERPQIPFVWKRPGEIVKNPEFILGGATRTDICQGELG
DCWLLAAIASLTLNQKALARVIPQDQSFGPGYAGIFHFQFWQHSEWLDVVIDDRLPTFRDRLVFLHSADHNEFWSALLEKAYAKLNG
SYEALKGGSAIEAMEDFTGGVAETFQTKEAPENFYEILEKALKRGSLLGCFIDTRSAAESEARTPFGLIKGHAYSVTGIDQVSFRGQ
RIELIRIRNPWGQVEWNGSWSDSSPEWRSVGPAEQKRLCHTALDDGEFWMAFKDFKAHFDKVEICNLTPDALEEDAIHKWEVTVHQ

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure: Using the SGC's LEX bubbling system, CAPN9 was expressed in E. coli BL21 (DE3) grown in Terrific Broth (TB) in the presence of 50 µg/mL of kanamycin at 37ºC to an OD_{600} of 7.5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.05 mM, and incubated overnight at 15ºC. The culture was centrifuged and the cell pellets were collected and stored at -80ºC.

Purification

Procedure

Extraction

Procedure

Frozen cell pellets contained in bags (Beckman 369256) obtained from 2L liters of culture are thawed by soaking in warm water for 5 minutes. Each cell pellet is resuspended in 20 mL lysis buffer (50 mM Tris pH 8.0 (VWR EM-9210), 500 mM NaCl (VWR EM-SX0420-1), 1mM EDTA), 1mM phenylmethanesulfonyl fluoride (Sigma P7626), 2mM CaCl2 and 1mL Sigma general protease inhibitor (Sigma P2714-1BTL, resuspended according to manufacturer's instructions) and then homogenized using an Ultra-Turrax T8 homogenizer (IKA Works) at maximal setting for 30-60 seconds per pellet. Cell lysis is accomplished by sonication (Virtis408912, Virsonic) on ice: the sonication protocol is 10 sec pulse at half-maximal frequency (5.0), 10 second rest, for 6 minutes total sonication time per pellet. Lysed cells are placed into centrifuge tubes (363647, Beckman Coulter) and centrifuged in a JA25.50 rotor in an Avanti J-20 XPI centrifuge (Beckman Coulter) for 20 minutes at 69,673 x g. The supernatant is decanted into a beaker, and the insoluble pellet discarded.

Concentration:

Ligand

MassSpec:

Crystallization: Purified CAPN9 was crystallized using the sitting drop vapor diffusion method. Diffracting crystals leading to the structure grew when the protein was mixed at 20 mg/mL with the reservoir solution (containing 18% Peg3350, 0.1M hepes pH 7.7, 0.2M $CaCl_2$) in a 1:1 volume ratio.

NMR Spectroscopy:

Data Collection:

Data Processing: