Capn13

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Revision

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Entry Clone Accession:NM_144575
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SGC Clone Accession:capn13.515.669:C2; plate SDC061 C2
Tag:
Host:

Construct

Prelude:
Sequence:

MGSSHHHHHHSSGLVPRGSDIDATQLQGLLNQELLTGPPGDMFSLDECRSLVALMELKVNGRLDQEEFARLWKRLVHYQHVFQKVQT
SPGVLLSSDLWKAIENTDFLRGIFISRELLHLVTLRYSDSVGRVSFPSLVCFLMRLEAMAKTFRNLSKDGKGLYLTEMEWMSLVMYN

Vector:

Growth

Medium:
Antibiotics:
Procedure:

Purification

Procedure
4 microL of clarified supernatant is reserved for later analysis by SDS-PAGE. The rest of the clarified supernatant is then diluted 1:2 in lysis buffer, and loaded at approximately 1mL/min by gravity onto 5 mL of Ni-NTA resin (Qiagen 30450). 5 column volumes of lysis buffer are used to wash the column at approximately 3 mL/min, followed by 5 column volumes of low imidazole buffer (lysis buffer + 10 mM Imidazole (VWR EM-5720) pH 8) at approximately 3 mL/min. A 4 microL sample of the low imidazole wash is saved for later analysis by SDS-PAGE. Samples are eluted from the Ni-NTA resin by exposure to 10 mL elution buffer (lysis buffer + 250 mM imidazole and 10% glycerol (EMD GX0185-5)) at 1mL/min flow rate. A 10 microL sample of the eluate is saved for SDS-PAGE analysis. 10 microL of each eluate is saved for measurement of protein concentration using Bradford reagent (BioRad 500-0202).

All gel filtration columns, buffers, and protocols are identical for uncut and thrombin-treated proteins. An XK 16x65 column (part numbers 18-1031-47 and 18-6488-01, GE Healthcare) packed with HighLoad Superdex 200 resin (10-1043-04, GE Healthcare) is pre-equilibrated with gel filtration buffer (lysis buffer + 5 mM ?-ME +1 mM EDTA + 2 mM CaCl2, all from Sigma) for 1.5 column volumes using an AKTAxpress (18-6645-05, GE Healthcare) at a flow rate of 3 mL/min. 5 mL of sample is loaded onto the column at 1.5 mL/min, and 2mL fractions are collected into 96-well plates (VWR 40002-012) using peak fractionation protocols with the following parameters: (Slope; min. peak width 0.833 min; level 0.000 mAU; peak start slope 10.000 AU/min; peak end slope 20.000 AU/min). Peak fractions are analyzed for purity using SDS-PAGE or visual analysis of the chromatogram and pooled.

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, 500 mM NaCl 2mM CaCl2), 1mM phenylmethanesulfonyl fluoride (Sigma P7626), and 1mL Sigma general protease inhibitor (Sigma P2714-1BTL, resuspended according to manufacturerÂ□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: Crystals of CAPN13 were obtained by means of hanging drop vapor diffusion crystallization. Crystals emerged at 298K when 10 mg/mL protein was mixed with25% PEG4K, 0.2M NH4SO4, 0.1M sodium acetate, pH 4.6.
NMR Spectroscopy:
Data Collection: The diffracting crystal was cryo-protected by 25% glycerol.Data were collected on the Rigaku FRE Superbright diffractometer at 1.54 Å.
Data Processing: