Human calpain 8

PDB:2NQA

Revision

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Entry Clone Accession:
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SGC Clone Accession:capn08a.023.346; plate SDC072 D11
Tag: Thrombin cleavable N-term his tag
Host:

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsNALKYLGQDFKTLRQQCLDSGVLFKDPEFPACPSALGYKDLGPGSPQTQGIIWKRPTELCPSPQFIVG
GATRTDICQGGLGDCWLLAAIASLTLNEELLYRVVPRDQDFQENYAGIFHFQFWQYGEWVEVVIDDRLPTKNGQLLFLHSEQGNEFW
SALLEKAYAKLNGCYEALAGGSTVEGFEDFTGGISEFYDLKKPPANLYQIIRKALCAGSLLGCSIDVYSAAEAEAITSQKLVKSHAY
SVTGVEEVNFQGHPEKLIRLRNPWGEVEWSGAWSDDAPEWNHIDPRRKEELDKKVEDGEFWMSLSDFVRQFSRLEICNLSPDSLS

Vector:

Growth

Medium:
Antibiotics:
Procedure: Inoculation: The appropriate well of a 96 well block from the -80oC freezer containing a glycerol stock of the bacteria of interest is pierced and plunged with a needle by a flame. The needle is dropped into a 250 ml flask containing 100 mL of LB (Sigma L7658) supplemented with 50 mg/ ml kanamycin (BioShop Canada KAN 201). The flask is shaken overnight (16 hours) at 250 rpm at 37oC. A 2L bottle (VWR 89000-242) containing 1800 ml of TB (Sigma T0918), supplemented with 1.5% glycerol, 50 mg/ ml kanamycin and 300 ul antifoam 204 (Sigma A-8311 is inoculated by a flame by pouring in the overnight LB culture. A sterilized cap/sparger (Fisher 11-138B) assembly is then inserted into the bottle.

Induction: The temperature of the media begins to be reduced to 15oC one hour prior to induction so that the media is at 15oC at the time of induction. Cultures are induced when the OD(600) reaches between 4 and 8 with 100 mM isopropyl-thio-b-D-galactopyranoside (BioShop Canada IPT 001), in the absence of flame. Cultures continue to be aerated overnight (16 hours) at 15oC. Aeration is slightly reduced at this time to avoid over foaming of the lower temperature culture.

Purification

Procedure

IMAC purification: The lysate is spun at 500xg for 3 minutes to pellet the HisLink resin. The lysate is carefully decanted off the resin, and then 50 mL of lysis buffer are added to wash the resin. The resin is allowed to settle for 5 minutes, then poured off and washed 3 more times with fresh lysis buffer. The washed resin is then loaded onto a gravity column, and then washed with a column volume of low imidazole buffer (lysis buffer + 30 mM imidazole pH 8). A 4 mL sample of the low imidazole wash is saved for later analysis by SDS-PAGE. Samples are eluted from the HisLink resin by exposure to 10 mL elution buffer (lysis buffer + 500 mM imidazole and 10% glycerol) at a 1mL/min flow rate. A 10 uL sample of the eluate is saved for SDS-PAGE analysis. 10 uL of each eluate is saved for measurement of protein concentration using Bradford.

(His)6-tag cleavage: 1 unit of thrombin protease per milligram of protein is added to the sample. The conical vial is stored without shaking, overnight, at 4oC.

Size exclusion chromatography: 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 b-ME and 1mM EDTA) for 1.5 column volumes using an AKTAxpress at a flow rate of 3 mL/min. 10 mL of sample is loaded onto the column at 1.5 mL/min, and 2mL fractions are collected into 96-well plates using peak fractionation protocols. Peak fractions are analyzed for purity using SDS-PAGE and/or mass spectrometry and pooled.

Protein concentration: Purified proteins are concentrated using either 4 mL or 15 mL concentrators with an appropriate molecular weight cut-off (Amicon Ultra-15 10,000 MWCO, UFC901024 or 5,000 MWCO, UFC900524, as appropriate, Millipore) to a final concentration of 20 mg/mL for crystallographic screening or other biophysical studies.

Extraction

Procedure

Frozen cell pellets contained in bags (Beckman 369256) obtained from 2L liters of culture are thawed by soaking in warm water. Each cell pellet is resuspended in 20 mL lysis buffer (50 mM Tris pH 8.0 and 500 mM NaCl 1mM phenylmethanesulfonyl fluoride and 1mL Sigma general protease inhibitor (Sigma P2714, resuspended according to manufacturerÂ□s instructions) and then homogenized at maximal setting for 30-60 seconds per pellet. Cell lysis is accomplished by sonication on ice: the sonication protocol is 10 sec pulse at half-maximal frequency, 10 second rest, for 6 minutes total sonication time per pellet. Lysed cells are diluted to 50 -100 mL final volume and imidazole added to a final concentration of 10 mM; this is then mixed with 2-3 mL of HisLink Protein Purification Resin (Promega V8821) per construct. The mixture is incubated with mixing for at least 20 minutes at 4oC.

Concentration:

Ligand

MassSpec:

Crystallization: Protein concentration: 10 mg/mL
Crystallization method: Sitting drop vapor diffusion
Crystallization conditions: 2M NH4PO4, 0.1M Tris, pH 8.5
Crystallization temperature: 18 degC

NMR Spectroscopy:

Data Collection:

Data Processing: