CAPN9 + leupeptin

PDB:2P0R

Revision

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Entry Clone Accession:AAB80762
Entry Clone Source:capn09.NM_006615.GSC.capn09.001.365.vec
SGC Clone Accession:capn09.010.340.02D05
Tag:N-terminal histag: mgsshhhhhhssglvprgs
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

gsPQAHPVPKDARITHSSGQSFEQMRQECLQRGTLFEDADFPASNSSLFYSERPQIPFVWKRPGEIVKNPEFILGGATRTDICQGEL
GDCWLLAAIASLTLNQKALARVIPQDQSFGPGYAGIFHFQFWQHSEWLDVVIDDRLPTFRDRLVFLHSADHNEFWSALLEKAYAKLN
GSYEALKGGSAIEAMEDFTGGVAETFQTKEAPENFYEILEKALKRGSLLGCFIDTRSAAESEARTPFGLIKGHAYSVTGIDQVSFRG
QRIELIRIRNPWGQVEWNGSWSDSSPEWRSVGPAEQKRLCHTALDDGEFWMAFKDFKAHFDKVEICNLTPDA

Vector:p28a-thrombin-LIC

Growth

Medium:TB
Antibiotics:
Procedure: Overnight cultures were grown from glycerol stocks in LB media. Large-scale inolculations were performed by adding 50 mLs of overnight culture into 900 mL of TB media supplemented with 1.5% glycerol, 50 µg/ ml kanamycin and 300 µL antifoam 204 (Sigma A-8311). Cells were grown at 37 °C using the LEX bubbling system until OD reaches ~5, at which time the temperature of the cultures were reduced to 15 °C. Cultures were induced when the cell density was between 6-8 with a final concentration of 100 µM IPTG and left overnight. Cells were harvested by centrifugation at 12,000xg for 15 minutes.

Purification

Procedure
Lysed cells were diluted 1:1 in lysis buffer; imidazole was added to a final concentration of 30 mM, and 1.5 mL of his-Link resin (Promega) was added to the lysate. This mixture was then rocked at 4 degC for 1 hour. After incubation for 1 hour, the mixture was centrifuged at 500xg and the supernatant carefully decanted. The resin was washed four times with IMAC buffer, then

resuspended and loaded into a gravity column. The resin was washed with 5 column volumes of low imidazole buffer and then eluted with 10 mLs of elution buffer. Two units of thrombin (Sigma) per milligram of protein were added and incubated overnight at 4 degC to cleave the (His)6 tag. The cut protein was then loaded onto an XK 16x65 column packed with highLoad Superdex 200 resin (GE Healthcare). Peak fractions were pooled and concentrated using Amicon concentrators with 10,000 MWCO (Millipore) to 20 mg/mL.

Extraction

Procedure
Frozen cell pellets were thawed on ice. Each pellet was resuspended in 25 mL lysis buffer, homogenized, and lysed using sonication.
Concentration:
Ligand
MassSpec:
Crystallization: Purified protein at a concentration of 10 mg/mL was incubated with 10-fold molar excess of leupeptin (Sigma) overnight. Crystals were obtained in hanging drop plates at 18 degC using the follow crystallization conditions: 14.7% PEG8000, 10% glycerol, 0.1M sodium cacodylate pH 5.5, and 0.15M $(NH_4)_2SO_4$. Crystals were harvested into cryoprotectant containing 15% glycerol and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection:
Data Processing: