Human UBH12

PDB:2FAZ

Revision

Revision Type:created
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Entry Clone Accession:ubh12.001.076.GSC.#.vec
Entry Clone Source:
SGC Clone Accession:ubh12.001.076; plate SDC029H12
Tag:MGSSHHHHHHSSGLVPR*GS
Host:E.coli BL21 (DE3)

Construct

Prelude:
Sequence:

MGSSHHHHHHSSGLVPR*GSMWIQVRTMDGRQTHTVDSLSRLTKVEELRRKIQELFHVEPGLQRLFYRGKQMEDGHTLFDYEVRLND
TIQLLVRQS

Vector:pET28a-LIC

Growth

Medium:
Antibiotics:
Procedure: The protein 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 OD600 of about 4. Cells were induced with 0.05 mM isopropyl-1-thio-D-galactopyranoside (IPTG), and incubated overnight at 15 ºC. The culture was centrifuged and cell pellets were collected and stored at -80ºC.

Purification

Procedure
The clarified supernatant from a 2 L culture was loaded at approximately 1 mL/min by gravity onto 4 mL of Ni-NTA resin (Qiagen 30450). Twelve column volumes of Wash buffer were used to wash the column at approximately 3 mL/min. Samples were eluted from the Ni-NTA resin by exposure to 7.5 mL Elution buffer at 1 mL/min flow rate. EDTA was added to the eluate to final concentration of 1 mM. Approximately 15 minutes later, dithiothreitol was added to the eluate to a final concentration of 2 mM. Protein concentration was determined using molecular coefficients. Thrombin (Sigma T9681; 1 unit per milligram of protein to 50 U maximum) and CaCl2 (4 mM final concentration) were added to the Ni-NTA eluate in 50 mL conical vials (352096, BD Biosciences) and the conical vial was incubated without shaking, overnight, at 4ºC. 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) was pre-equilibrated with Gel filtration buffer using an AKTA Purifier. The full volume of eluate was loaded onto the column at 1.5 mL/min and 2 mL fractions collected using peak fractionation protocols. Peak fractions were analyzed for purity using SDS-PAGE or visual analysis of the chromatogram and pooled. The protein was concentrated by ultrafiltration and analyzed by mass spectrometry.

Extraction

Procedure
The cell pellet from a 2 L culure was resuspended in Lysis buffer and lysed using Microfluidizer. Fresh PMSF was added to the lysate and the lysate cleared by centrifugation (24,000 rpm, 30 minutes).
Concentration:
Ligand
MassSpec:
Crystallization: Purified protein was crystallized using the sitting drop vapor diffusion method. Crystals grew when the protein (13 mg/mL) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop was equilibrated against a reservoir solution containing 20.0% polyethylene glycol 3350, 0.2 M tri-Lithium Citrate in 291 K temperature.
NMR Spectroscopy:
Data Collection:
Data Processing: