GRLF1

PDB:3FK2

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source:CDV synthesized: SGC:25-E5
SGC Clone Accession:HPC095-A04
Tag:N-terminal tag: mhhhhhhssgrenlyfq*g
Host:BL21(DE3)-V2R-pRARE2

Construct

Prelude: Tag not removed. GRLF1:D1212-R1439
Sequence:

mhhhhhhssgrenlyfqgDPRRRNILRSLRRNTKKPKPKPRPSITKATWESNYFGVPLTTVVTPEKPIPIFIERCIEYIEATGLSTE
GIYRVSGNKSEMESLQRQFDQDHNLDLAEKDFTVNTVAGAMKSFFSELPDPLVPYNMQIDLVEAHKINDREQKLHALKEVLKKFPKE
NHEVFKYVISHLNKVSHNNKVNLMTSENLSICFWPTLMRPDFSTMDALTATRTYQTIIELFIQQCPFFFYNR

Vector:pET28-MHL (GI:134105571)

Growth

Medium: Terrific Broth
Antibiotics: Kanamycin 50 µg/mL Chloramphenicol 25 µg/mL
Procedure:LEX Bubbling. The target protein was expressed in E. coli by inoculating 60 mL of overnight culture grown in Luria-Bertani medium into a 1.8 L of Terrific Broth medium in the presence of 50 µg/mL kanamycin and 25 µg/mL Chloramphenicol at 37 degC. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 degC and the culture was induced with 0.5 mM IPTG. The cells were allowed to grow overnight before they were harvested and flash frozen in liquid nitrogen and stored at -80 degC.

Purification

Procedure
The lysate was centrigued at 15,000 rpm for 45 minutes and the supernatants were mixed with 3 mL 50% Ni-NTA beads, and incubated at 4 degC for 1 hours. The supernatant was then passed through a gravity column (Poly-Prep, Bio-Rad, Catalog #731-1550) and the beads were washed using 15 mL washing buffers(contains 5mM, 30mM or 75 mM Imidazole separately). The protein bound to beads were eluted using 15 mL elution buffer once. The flow-through fractions washed using buffer containing 30mM, 75mM, 300mM Imidazole were collected and loaded onto

Supderdex-75 gel filtration column. Eluted fractions were pooled and concentrated using amicon centrifugal filter (m.w. cut-off 10,000). The purity of the proteins was higher than 95% judged by SDS-PAGE.

Extraction

Procedure
Frozen cells from 1.8L TB culture were thawed and resuspended in 150 mL extraction buffer with freshly added 0.5% CHAPS and 2mM BME, and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 3 µL benzonase (Sigma Catalog # E1014, 250U/µL), and lysed using microfluidizer.
Concentration:31.2 mg/mL
Ligand
MassSpec: Native: 28835.60, expected 28834.98
Crystallization: Initial crystallization screen was setup using sitting drops with Red Wings and SGC-I screens and also with in situ proteolytic treatment. Plate-shaped crystals were seen at multiple conditions with in situ chymotrypsin or trypsin treatment, include Red Wings condition: A7, B7, C10, D10, H2 for Chymotrypsin, and F7, F9, G7, H4 for Trypsin.

Crystal for data collection was grown in Plate (HPZ12Q-E8#2, OptRH4-96), Cryo-protectant used 0.8V well solution plus 0.2V 80% Glycerol.
The crystal(frozen pkey=206254) was grown in 26.4% PEG3350, 0.1M Bis-Tris pH 6.0, 0.2M Li2SO4, 1mM DTT, 0.5 uL protein in gel filtration buffer plus 0.5 uL well solution and 1:100 (w/w) Trypsin, in sitting drop setup.
NMR Spectroscopy:
Data Collection:
Data Processing: