PUS7

PDB:5KKP

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC011396
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: 6XHis-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQ*G
Host:E.coli BL21 (DE3) codon plus RIL (Stratagen).

Construct

Prelude:
Sequence:

gESFADMMKHGLTEADVGITKFVSSHQGFSGILKERYSDFVVHEIGKDGRISHLNDLSIPVDEEDPSEDIFTVLTAEEKQRLEELQL
FKNKETSVAIEVIEDTKEKRTIIHQAIKSLFPGLETKTEDREGKKYIVAYHAAGKKALANPRKHSWPKSRGSYCHFVLYKENKDTMD
AINVLSKYLRVKPNIFSYMGTKDKRAITVQEIAVLKITAQRLAHLNKCLMNFKLGNFSYQKNPLKLGELQGNHFTVVLRNITGTDDQ
VQQAMNSLKEIGFINYYGMQRFGTTAVPTYQVGRAILQNSWTEVMDLILKPRSGAEKGYLVKCREEWAKTKDPTAALRKLPVKRCVE
GQLLRGLSKYGMKNIVSAFGIIPRNNRLMYIHSYQSYVWNNMVSKRIEDYGLKPVPGDLVLKGATATYIEEDDVNNYSIHDVVMPLP
GFDVIYPKHKIQEAYREMLTADNLDIDNMRHKIRDYSLSGAYRKIIIRPQNVSWEVVAYDDPKIPLFNTDVDNLEGKTPPVFASEGK
YRALKMDFSLPPSTYATMAIREVLKMDTSIKNQTQLNTTWLR

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:PUS7 protein was expressed in E.coli BL21 (DE3) codon plus RIL in M9 minimum medium in the presence of 50 μg/ml of kanamycin. Cell were grown at 37oC to an OD600 of 0.8 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, in the presence of 50 mg/L of SeMet and incubated overnight at 15oC.

Purification

Buffers
Procedure
The crude extract was cleared by centrifugation. The lysate was loaded onto 5 ml HiTrap column (GE Healthcare), charged with Ni2+. The column was washed with 10 CV of 20 mM HEPES pH 7.4, containing 500 mM NaCl, 50 mM imidazole, 5% glycerol, and the protein was eluted with elution buffer (20 mM HEPES pH 7.5, 500 mM NaCl, 250 mM imidazole, 5% glycerol). The

protein was loaded on Superdex200 column (26x60) (GE Healthcare), equilibrated with 20 mM PIPES, pH 6.5, 250 mM NaCl. The fractions containing PUS7 were pooled and TEV protease was added to remove His-tag. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30S column (10x10) (GE Healthcare), equilibrated with buffer containing 20 mM PIPES, pH 6.5, and eluted with linear gradient of NaCl up to 500 mM concentration (20 CV).

Extraction

Buffers
Procedure
Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80˚C. For purification, the cell paste was thawed and resuspended in lysis buffer buffer (50 mM HEPES, pH 7.4, 0.5 M NaCl, 5 mM imidazol, 2 mM β-mercaptoethanol, 5% glycerol) with protease inhibitor (1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:24.7 mg/mL - Enzymatic treatment: TEV
Ligand
MassSpec: The expected mass for PUS7 is 64415 Da, measured mass for SeMet protein is 65214 Da.
Crystallization: Purified PUS7 protein (10.1 mg/mL) was crystallized using sitting drop vapor diffusion method at 20 °C by mixing 1 µl of the protein solution with 1 µl of the reservoir solution containing 20% PEG3350, 0.2 M sodium formate, pH 7.0
NMR Spectroscopy:
Data Collection:
Data Processing: