ZNF289

PDB:2P57

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_115765
Entry Clone Source:
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with thrombin cleavage site: mgsshhhhhhssglvprgs
Host:E.coli BL21 (DE3) codon plus RIL

Construct

Prelude:
Sequence:

mhhhhhhssglvprgsAEPNKTEIQTLFKRLRAVPTNKACFDCGAKNPSWASITYGVFLCIDCSGVHRSLGVHLSFIRSTELDSNWN
WFQLRCMQVGGNANATAFFRQHGCTANDANTKYNSRAAQMYREKIRQLGSAALARHG

Vector:pET28a-LIC

Growth

Medium: Terrific Broth
Antibiotics:
Procedure: The target was expressed in E. coli by inoculating 100 mL of overnight culture grown in Luria-Bertani medium into a 1.8 L of Terrific Broth medium in the presence of 50 microG/mL kanamycin and chloramphenicol at 37degC. When OD600 was ~3.0, the culture was induced with 1mM IPTG and the temperature was reduced to 15degC, and the cells were allowed to grow overnight before harvesting and flash frozen.

Purification

Procedure
The thawed cell pellets were resuspended in 100 mL of the binding buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM mercaptoethanol (BME)) with a protease inhibitor cocktail (0.1 mM M benzamidine-HCl and 0.1 mM phenylmethyl sulfonyl fluoride), and 0.5% CHAPS. The cells were lysed by liquid fluidizer. The lysate was centrifuged at 15000 rpm for 45 min and the supernatant was loaded onto 5 mL Ni-NTA column (Qiagen) equilibrated with the same binding buffer at 4 degC. The Ni-NTA column was washed with 150 mL of the wash buffer (20mM HEPES pH 7.5, 150 mM NaCl, 30 mM imidazole, 1mM BME) and the protein was eluted with 15 mL of the elution buffer (20mM HEPES pH 7.5, 150 mM NaCl, 250 mM imidazole, 1 mM

BME). The protein were further purified and desalted using gel filtration column, Superdex 75 (26/60), which was pre-equilibrated with the binding buffer. Collected fractions were concentrated using an Amicon Ultra centrifugal filter to a final concentration of 50 around mg/mL Protein concentrations were measured using Bradford assay with purity >95% based on SDS-PAGE analysis.

Extraction

Procedure
The thawed cell pellets were resuspended in 100 mL of the binding buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 1 mM mercaptoethanol (BME)) with a protease inhibitor cocktail (0.1 mM M benzamidine-HCl and 0.1 mM phenylmethyl sulfonyl fluoride), and 0.5% CHAPS. The cells were lysed by liquid fluidizer.
Concentration:30 mg/mL
Ligand
MassSpec:
Crystallization: Crystallization trials were set up using the sitting drop vapor diffusion method. The protein drop was equilibrated against a reservoir solution (1:1 volume ratio) containing 3.2 M Sodium Formate, 90 mM Sodium Acetate, pH 4.6. Crystals reached a size of about 50 microns within two to three days.
NMR Spectroscopy:
Data Collection:
Data Processing: