DDX18

PDB:3LY5

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|38327634
Entry Clone Source:BC024739
SGC Clone Accession:DDX18A-s002
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*sm
Host:*E.coli* BL21(DE3) gold pRARE2, supplies tRNAs for rare codons.

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfq*smNNVEKPDNDEDESEVPSLPLGLTGAFEDTSFASLCNLVNENTLKAIKEMGFTNMTEIQHKSIR
PLLEGRDLLAAAKTGSGKTLAFLIPAVELIVKLRFMPRNGTGVLILSPTRELAMQTFGVLKELMTHHVHTYGLIMGGSNRSAEAQKL
GNGINIIVATPGRLLDHMQNTPGFMYKNLQCLVIDEADRILDVGFEEELKQIIKLLPTRRQTMLFSATQTRKVEDLARISLKKEPLY
VG

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Cells from a glycerol stock were grown in 50 mL TB supplemented with 8 g/l glycerol, 100 µg/mL kanamycin and 34 µg/mL chloramphenicol at 37 °C overnight. The overnight culture (50 mL) was used to inoculate 3 l TB (divided into 4 x 0.75 l bottles) supplemented with 8 g/l glycerol, 50 µg/mL kanamycin and approximately 200 µl 204 Antifoam A6426 (Sigma) per bottle. The culture was grown in a LEX bioreactor system (Harbinger Biotechnology) at 37 °C until OD_{600} nm had reached 1-2. The culture was down-tempered to 18 °C over a period of 1 hour before target expression was induced by addition of 0.5 mM IPTG. Expression was allowed to continue overnight and cells were harvested the following morning by centrifugation (4,400 x *g*, 10 min, 4 °C). The resulting cell pellet (65 g wet cell weight) was then stored at -80 °C.

Purification

Procedure

Columns

IMAC: Ni-charged 5 mL HisTrap HP (GE Healthcare)Gel filtration column: HiLoad 16/60 Superdex 75 Prep Grade (GE Healthcare)

Procedure

The whole purification procedure was performed at 4 ºC. The protein sample was loaded onto an IMAC column previously equilibrated in Lysis buffer. The IMAC column was then washed with, first, 100 mL of IMAC wash1 buffer followed by 100 mL of IMAC wash2 buffer. Bound protein was eluted from the IMAC columns with IMAC elution buffer then loaded onto the gel filtration column equilibrated in GF buffer. Fractions containing the target protein were pooled.

Extraction

Procedure
1.5 ml Extraction buffer per gram cell pellet and the 1 ml per 1.5 L culture Complete stock solution[1] was added and the cell pellets were resuspended at 4°C. The resuspended cells were stored in falcon tubes in the -80 freezer. Cells were disrupted by sonication (Vibra-Cell, Sonics) at 80% amplitude for 3 min effective time (pulsed 4s on, 4s off) and cell debris was removed by centrifugation (49,000 x *g*, 40 min, 4 ºC). The supernatant was decanted and filtered through a 0.45 µm flask filter.

[1]Complete stock solution: 1 tablet Complete EDTA-free (protease inhibitor) and 8 µL Benzonase (2000 U) dissolved in 1 mL buffer
Concentration:
Ligand
MassSpec:
Crystallization: The Protein solution was incubated with 10 mM ADP and 10 mM MgCl2 for 1 hour. Crystals were obtained by the sitting drop vapour diffusion method in a 96-well plate. 0.1 µl protein solution (16.9 mg/ml) was mixed with 0.1 µl of well solution consisting of 0.1 M HEPES pH 6.6, 0.2 M sodium thiocyanate, 20% w/v PEG 3350. The plate was incubated at 20 ºC and crystals appeared after 14 days. The crystals were quickly transferred to a cryo solution consisting of 1 M HEPES pH 6.6, 0.2 M sodium thiocyanate, 22% w/v PEG 3350, 20% glycerol and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Diffraction data to 2.7 Å resolution was collected at Diamond beamline I04.
Data Processing: Intensity integration was performed by MOSFLM. Reflections were scaled with SCALA. The space group was P31 with cell dimensions a = 41.34 Å, b = 41.34 Å c = 230.54 Å, alpha = 90°, beta = 90°, gamma = 120°. The structure was solved by molecular replacement using MOLREP with the structure of human DEAD-BOX RNA helicase DDX10 as template (PDB: 2PL3). Structure refinement was performed with REFMAC 5.5.0102. R-factor = 0.246, R-free = 0.274.