TDRD2

PDB:3FDR

Revision

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Revised by:created
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Entry Clone Accession:NP_006853
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SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgrenlyfq*g. The tag was removed for crystallization.
Host:*E. coli* BL21(DE3)-V2R-pRARE2

Construct

Prelude:
Sequence:

gGSRSLQLDKLVNEMTQHYENSVPEDLTVHVGDIVAAPLPTNGSWYRARVLGTLENGNLDLYFVDFGDNGDCPLKDLRALRSDFLSL
PFQAIECS

Vector:pET28-MHL

Growth

Medium:TB
Antibiotics:
Procedure:A fresh transformation was used to inoculate 20 mL LB media containing 50 µg/mL kanamycin. The culture was grown overnight at 37°C with shaking. The next day this starter culture was used to innoculate 2L of TB medium which contained 50 µg/mL kanamycin. The culture was grown in LEX at 37°C to OD600 of 2.3 and was induced with the addition of 0.5 mM IPTG. The temperature was reduced to 16°C and the culture was incubated for a further 18 hours before harvesting the cells.

Purification

Procedure
Column 1: Affinity purification, open Ni-NTA column Procedure: The supernatant was incubated with 6mL of 50% slurry Ni-NTA beads by rocking. After 1 hour incubation at 4ºC, the beads were washed with 50 mL of lysis buffer. The protein was eluted using ~20mL EB.

Column 2: HiTrap Q HP 5mL Procedure: The eluent from the Ni column diluted 1:20 in Buffer A and manually loaded on the column. The protein was then eluted via a linear gradient from 0 -

100% of buffer B.

Column 3: Gel filtration, HiLoad 16/60 Superdex 75 Prep Grade Procedure: The eluent from from the IEX column was loaded onto the gel filtration column in GF buffer at 1 mL/min, fraction size 2mL. The fractions containing protein were identified on a SDS-PAGE gel. Final step: The Histidine tag was removed via incubated with TEV protease and the resulting sample purified using the same conditions as column 3.

Extraction

Procedure
Cells were harvested by centrifugation and pellets were stored in -80ºC. Prior to purification, the cell pellet was resuspended in lysis buffer. Cells were disrupted by sonication (10 minutes) and samples were centrifuged for 60 min at 70000 g.
Concentration:20.25 mg/ml.
Ligand
MassSpec:
Crystallization:30% PEG 4000, 0.2 M Ammonium Acetate, 0.1 M NaCitrate, pH 5.6
NMR Spectroscopy:
Data Collection:
Data Processing: