TDRD3

PDB:3PMT

Revision

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Tag:N-terminal tag: MGSSHHHHHHSSRENLYFQG
Host:*E. coli* BL21(DE3)-V2R-pRARE2

Construct

Prelude:
Sequence:

KMWKPGDECFALYWEDNKFYRAEVEALHSSGMTAVVKFIDYGNYEEVLLSNIKPIQTE

Vector:pET28-MHL

Growth

Medium:TB media
Antibiotics:50 µg/mL kanamycin and 30 µg/mL chloramphenicol
Procedure:A fresh transformation was used to inoculate 60 mL LB media containing 50 µg/mL kanamycin and 30 µg/mL chloramphenicol. The culture was grown overnight at 37°C with shaking. The next day this starter culture was used to inoculate 2L of TB growth medium containing 50 µg/mL kanamycin and 30 µg/mL chloramphenicol. The culture was grown in LEX at 37°C to OD_{600} of 2.5. The temperature was reduced to 14°C and IPTG-based induction (1mM) was carried out according to the manufacturer's protocol. The culture was incubated for a further 18 hours before harvesting the cells. Cells were harvested by centrifugation and pellets were stored at -80°C.

Purification

Procedure
Column 1: Affinity purification, open Ni-NTA column
Procedure: The supernatant was incubated with 6mL of 50% slurry Ni-NTA beads (buffer is changed to lysis buffer prior to use) by rocking. After 1 hour incubation at 4ºC, the bead mixture was transferred to an empty column and washed with wash buffer, then using 15ml elution buffer elute.
Parts of eluted sample were treated with V8 and TEV which was using for crystallization.

Column 2: Size Exclusion, HiLoad 16/60 Superdex 75 Prep Grade
Procedure: The elution from the NiNTA column and treated protein were concentrated using 15 mL concentrators with a 10K Da molecular weight cut-off (Amicon Ultra-15, Millipore). The concentrated protein was loaded onto the size exclusion column at a flow rate of 1 mL/min, and 2 mL fractions were collected. The fractions containing protein were identified on an SDS-PAGE gel. Pool the fractions together and concentrated it using the 15 mL concentrators with a 10K Da molecular weight cut-off (Amicon Ultra-15, Millipore).

Extraction

Procedure
Prior to purification, the cell pellet was resuspended in lysis buffer. Cells were disrupted by sonication (100 watts, 10 minutes total time using 10 second pulses followed by 10 second rest) on ice and samples were centrifuged for 60 min at 16000 RPM.
Concentration: The final concentration was 10 mg/ml. The protein yield was approximately 40 mg per liter of bacterial culture.
Ligand
MassSpec:
Crystallization: Protein used for crystallization is his-tag free. Pool together the homogeneous fraction and cleave his-tag by adding TEV and incubate at 4°C for overnight. The removed his-tag protein re-binds with Ni-NTA and protein elution goes through exclusion column once more (20mM Tris pH7.0, 200Mm, 1mM DTT). Protein was concentrated to 10mg/ml and crystallize by sitting-drop vapor diffusion, 0.5 ul protein mixing with 0.5 ul crystallization buffer (0.1M Tris-HCl pH 8.5, 2.0M Ammonium sulfate), temperature 18°C.
NMR Spectroscopy:
Data Collection:
Data Processing: