TDRKH

PDB:5J39

Revision

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Entry Clone Accession:AT54-G12
Entry Clone Source:
SGC Clone Accession:JMC107-A1
Tag:N-terminal His6-tag
Host:BL21(DE3)CodonPlus RIL

Construct

Prelude:
Sequence:

MHHHHHHSSGRENLYFQGEVYVSASEHPNHFWIQIVGSRSLQLDKLVNEMTQHYENSVPEDLTVHVGDIVAAPLPTNGSWYRARVLG
TLENGNLDLYFVDFGDNGDCPLKDLRALRSDFLSLPFQAIECSLARIAPSGDQWEEEALDEFDRLTHCADWKPLVAKISSYVQTGIS
TWPKIYLYDTSNGKKLDIGLELVHKGYAIELPED

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:A fresh transformation was used to inoculate 50 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. The culture was grown in LEX at 37°C to OD600 of 1. IPTG-based induction was carried out according to the manufacturer's protocol. The temperature was reduced to 18°C and the culture was incubated for a further 18 hours before harvesting the cells.

Purification

Buffers
Washing Buffer: 50 mM Tris-HCl pH 7.6, 500 mM NaCl, 5% glycerol, 1 mM imidazole - Elution Buffer: 50 mM Tis-HCl pH 7.6, 500 mM NaCl, 5% Glycerol, 250 mM imidazole
Procedure
The N-terminal His-tagged fusion proteins were purified by affinity chromatography on Ni-NTA agarose resin (Qiagen). The elution fraction was collected and purified further by size exclusion

chromatography (Superdex 200; GE Healthcare). The proteins were concentrated to 40 mg/mL in a buffer containing 20 mM Tris-HCl, pH 8, 200 mM NaCl, 5% glycerol and 1 mM DTT.

Extraction

Buffers
50 mM Tris-HCl pH 7.6, 500 mM NaCl, 5% Glycerol
Procedure
2L native cell pellet was resuspended in a total volume of 200 mL extraction buffer with1mM PMSF/Benzamidine freshly added and the cells disrupted by sonication for 10 mins at 6" on 8" off duty cycle at 120W output power.
Concentration: Concentration used for crystallization : native protein: 30-40 mg/mL
Ligand
MassSpec:
Crystallization: Crystal screenings were performed by sitting-drop vapor-diffusion methods at 18 °C. Crystals were grown by mixing 1 µl of protein with 1 µl of reservoir solution (0.2 M magnesium chloride, 0.1 M sodium cacodylate, pH 5.5, 15% (w/v) PEG8000).
NMR Spectroscopy:
Data Collection:
Data Processing: