TULP1

PDB:2FIM

Revision

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Entry Clone Accession:gi|21619193
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Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgvdlgtenlyfq*s(m).
Host:E. coli Bl21(DE3)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsMEPREFVLRPAPQGRTVRCRLTRDKKGMDRGMYPSYFLHLDTEKKVFLLAGRKRKRSKTANYLIS
IDPTNLSRGGENFIGKLRSNLLGNRFTVFDNGQNPQRGYSTNVASLRQELAAVIYETNVLGFRGPRRMTVIIPGMSAENERVPIRPR
NASDGLLVRWQNKTLESLIELHNKPPVWNDDSGSYTLNFQGRVTQASVKNFQIVHADDPDYIVLQFGRVAEDAFTLDYRYPLCALQA
FAIALSSFDG

Vector:pNIC-Bsa4

Growth

Medium:
Antibiotics:
Procedure:30 µL competent Bl21(DE3) cells (Novagen) were transformed with 1 µL plasmid. Held 30min on ice, heat-shocked in 42 degree waterbath for 45sec at 42°C. Held on ice for 2 min. Added 100µL SOC and incubated in shaker for 1 h. Cells were plated on LA plates with 50 mg/l Kanamycin and 0.2% glucose. One colony was used to inoculate 20 ml of TB + Kan 50 mg/l. The inoculation culture was shaken at 30 degrees overnight. The inoculation culture was added to two TunAir flask (Shelton Scientific) with 750 ml of phosphate buffered TB with 50 mg/l Kanamycin. The culture was incubated at 37°C, until OD600 reached of approximately 1.2. The temperature was lowered to 18°C and the culture was induced with 0.5 mM IPTG for 18 hours.

Purification

Procedure
Buffers: 50 mM Na-Phosphate, 500 mM NaCl, 10% glycerol, 10 mM imidazole, 0.5 mM TCEP (IMAC Bind/Wash1 Buffer); 50 mM Na-Phosphate, 500 mM NaCl, 10% glycerol, 25 mM

imidazole, 0.5 mM TCEP (IMAC Wash2 Buffer); 50 mM Na-Phosphate, 500 mM NaCl, 10% glycerol, 500 mM imidazole, 0.5 mM TCEP (IMAC Elution Buffer). 20 mM HEPES pH 7.5, 300 mM NaCl, 10% glycerol, 0.5 mM TCEP (Gelfiltration buffer).

Columns: 1 ml Hi-Trap Chelating (Ni-charged). (GE Healthcare). Superdex 200 HiLoad 16/60 (GE Healthcare).
Procedure: The sample was purified automatically on an ÄKTA-Xpress (GE Healthcare). Briefly, sample was loaded on the IMAC column, eluted in a storage loop and then loaded on the gel filtration column. Elution fractions were pooled based on SDS-PAGE analysis. Protein was estimated by SDS-PAGE analysis to be more than 95% pure. Fresh TCEP was added to the pooled samples so that the concentration of TCEP was 2 mM. Concentration was performed by use of Amicon Ultra 15 (Millipore) with 10 000 MW CO. Centrifugation was performed at 15 deg in swing-out buckets at 3000 g. Yield of purified protein per liter of culture was 7.1 mg.

Extraction

Procedure
Cells were harvested by centrifugation (WCW 31.5 g) and pellets were resuspended in 60 ml of lysis buffer (50 mM Na-Fosfate, 500 mM NaCl, 10% glycerol, 10 mM imidazole, 0.5 mM TCEP and 1 tablet Complete EDTA-free protease inhibitor (Roche Biosciences)). After thawing, 4 µl of a 250 U/µl benzonase (Novagen) stock solution was added and lysis buffer was added to a total volume of 70 ml. Cells were then disrupted by high pressure homogenization with a high-pressure homogenizer (Stansted) (4 passes) prior to centrifugation for 30 min at 49000 g in a Sorvall SA-800 rotor. The soluble fraction was decanted and filtered through 0.45 µm.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained using the sitting drop method at 20°C. Drops were prepared using 1 µl of protein (10.7 mg/ml concentration) and 1 µl of the well solution (0.2 M Ammonium sulphate, 25% Peg 4000, 15 % Glycerol and 50 mM 3-(N,N-dimethyloctylammonio)propanesulfonate (FLUKA)). Small plate- shaped crystals grew overnight.
NMR Spectroscopy:
Data Collection:
Data Processing: