UBA5

PDB:3GUC
Entry Clone Source:uba05.BC009737.MGC.AT9-F4.pCMV-SPORT6
SGC Clone Accession:uba05.057.329.165G11 (SDC165G11)
Tag:N-terminal tag: MGSSHHHHHHSSGLVPRGS
Host:BL21 (DE3)
Vector:pET28a-LIC

Sequence (with tag):
mgsshhhhhhssglvprgsMALKRMGIVSDYEKIRTFAVAIVGVGGVGSVTAEMLTRCGIGKLLLF
DYDKVELANMNRLFFQPHQAGLSKVQAAEHTLRNINPDVLFEVHNYNITTVENFQHFM
DRISNGGLEEGKPVDLVLSCVDNFEARMTINTACNELGQTWMESGVSENAVSGHIQLIIP
GESACFACAPPLVVAANIDEKTLKREGVCAASLPTTMGVVAGILVQNVLKFLLNFGTVSF
YLGYNAMQDFFPTMSMKPNPQCDDRNCRKQQEEYKKKVAALPKQEVIQ

Growth

Procedure: Media bottles (2L) containing TB (Sigma T0918) supplemented with 1.5% glycerol, 50 µg/ml kanamycin and 600 µl antifoam 204 (Sigma A-8311) were inoculated with 50-100 ml of the overnight LB culture each. With sterilized cap/sparger (Fisher 11-138B) assemblies, bottles were placed into a circulating water bath set at 37 degC. Temperature was reduced to 15 degC one hour prior to induction at OD600 between 4 and 8 with 100 µM isopropyl-thio-B-D-galactopyranoside (BioShop Canada IPT 001). Cultures were aerated overnight (16 hours) at 15 degC.

Purification

Procedure: Four milliliters of TALON metal-affinity resin (BD Bioscience) was mixed for 2 hours at 4 degC with 150 mL lysate, centrifuged for 3 minutes (SX4750 rotor, Allegra X-12R, Beckman Coulter), and decanted. Beads were transferred into a 25 mL Econo-Column (Bio-Rad 732-1010) and washed with 3 x 15 mL Wash buffer. Sample was eluted with 3 column volumes of Elution buffer. Samples were gel-filtered (XK 16x65 packed with HighLoad Superdex 200 resin, GE Healthcare) using an AKTAxpress (18-6645-05, GE Healthcare) at a flow rate of 1 mL/min Gel-filtration buffer, and 3 mL fractions were collected in 5 ml tubes. Fractions containing protein were pooled and centrifuged through concentrators with 10,000 kDa cut-off (Amicon Ultra-15, UFC900524, Millipore) for 45 minutes at 3750 rpm.

Extraction

Procedure: Frozen cell pellets contained in bags (Beckman 369256), obtained from 2L cultures, were thawed by soaking in warm water, and resuspended in 25-40 mL Lysis buffer. Cell lysis was accomplished by sonication on ice (Virtis408912, Virsonic: 10 sec, 50 % power, 10 sec rest, 10 min total sonication time per pellet).
Concentration:24 mg/mL

Structure Determination

Crystallization: Crystals of Uba5 were grown at 287 K using the hanging drop method by mixing equal volumes of 0.8 M Lithium sulfate, 0.5 M Ammonium sulfate, 0.1 M Sodium Citrate pH 6.2

and 24 mg/mL protein in gel filtration buffer plus 5 mM AMPPNP and 5 mM $MgCl_2$. The crystals were cryoprotected by dragging the crystal through a drop containing cryoprotectant solution (9% sucrose (wt/vol), 2% glucose (wt/vol), 8% glycerol (vol/vol), 8% ethylene glycol (vol/vol)) and reservoir buffer.