

UBA5

PDB:3H8V

Entry Clone Source:uba05.BC009737.MGC.AT9-F4.pCMV-SPORT6

SGC Clone Accession:uba05.057.329.165G11 (SDC165G11)

Tag:N-terminal tag: MGSSHHHHHSSGLVPRGS

Host:BL21 (DE3)

Vector:pET28a-LIC

Construct

Sequence:

mgsshhhhhhsglprgsMALKRMGIVSDYEKIRTFAVAIVGVGGVGSVTAEMLRCGIGKLLFDYDKVELANMNRLFFQPHQAGLSKVQAAEHTLRNINPDVLFEVHNYNITTVENFQHFMDRISNGGLEEGKPVDLVLSCVDNFEARMINTACNELGQTWMESGVSENAVSGHIQLIIPGESACFACAPPLVVAANIDEKTLKREGVCAASLPTTMGVVAGILVQNVLKFLLNFGTVSFYLGYNAMQDFFPTMSMKPNPQCDDRNCRKQQEYKKVAALPKQEVIQ

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-β-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 4oC 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:20 mg/mL

Structure Determination

Crystallization: Crystals of Uba5 were grown at 287 K using the hanging drop method by mixing equal volumes of 1 M Lithium sulfate, 0.3 M Ammonium sulfate, 0.1 M Sodium Citrate pH 6.2 and 20 mg/mL protein solution in gel filtration buffer. 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.