

Human UBCi

PDB:1ZUO

Revision

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Entry Clone Accession:gi:29789400

Entry Clone Source:MGC

SGC Clone Accession:ubc63.197.363; plate SDC007C:9

Tag:N-terminal His-tag with integrated thrombin-cleavage site MGSSHHHHHSSGLVPRGS.

Host:E.coliBL21 (DE3)

Construct

Prelude:

Sequence:

MGSSHHHHHSSGLVPRGSGAVGSVQASDRLMKELRDIYRSQSYKTGIYSVELINDSLYDWHVKLQVDPDSPLHSDLQILKEKEG
IEYILLNFSFKDNFPFDPPFVRVLPVLSGGYVLGGGALCMELLTKQGWSSAYSIESVIMQINATLVKGKARVQFGANKQYNLARA
QQSYNSIVQIHE

Vector:p28a-LIC

Growth

Medium:

Antibiotics:

Procedure:UBCi was expressed in E. coli BL21 (DE3) grown in Terrific Broth (TB) in the presence of 50 microG/ml of kanamycin at 37degC to an OD600 of 7.5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.05 mM, and incubated overnight at 15°C. The culture was centrifuged and the cell pellets were collected and stored at -80°C.

Purification

Procedure

The cleared lysate was loaded onto a TALON metal-affinity resin column from BD Biosciences at 4degC. The column was washed with wash buffer A (10mM Tris-HCl pH 8.0, 0.5 M NaCl, 5% glycerol, 10 mM imidazole, 1 mM β -mercaptoethanol), wash buffer B (same as wash buffer A but containing 0.05% Tween with protease 20) and again wash buffer A, and the protein was eluted with 10 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 200 mM imidazole, 1 mM β -mercaptoethanol. The protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with 20 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 2mM dithiothreitol (DTT) and concentrated by ultrafiltration.

Extraction

Procedure

The cell pellet was resuspended in lysis buffer (10 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 2 mM imidazole, 1 mM β -mercaptoethanol) inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF) and lysed using Microfluidizer. The lysate was cleared by centrifugation.

Concentration:

Ligand

MassSpec:

Crystallization: Purified UBCi was crystallized using the hanging drop vapor diffusion method. Crystals grew when the protein (12 mg/mL) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop was equilibrated against a reservoir solution containing 32% PEG1500, 0.2 M NaCl, 0.1 M Na Cacodylate at pH 5.8 in 293K temperature.

NMR Spectroscopy:

Data Collection:

Data Processing: