Human UBE2E2

PDB:1Y6L

Revision

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Revised by:created
Revision Date:created
Entry Clone Accession:gi:18490186
Entry Clone Source:MGC
SGC Clone Accession:ubc46.055.201; plate SDC006:C2
Tag:N-terminal His-tag with integrated thrombin-cleavage site MGSSHHHHHHSSGLVPRGS.
Host:E.coli BL21 (DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsTSAKRIQKELAEITLDPPPNCSAGPKGDNIYEWRSTILGPPGSVYEGGVFFLDITFSPDYPFKPPKVT
FRTRIYHCNINSQGVICLDILKDNWSPALTISKVLLSICSLLTDCNPADPLVGSIATQYMTNRAEHDRMARQWTKRYAT

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure:

Purification

Procedure
The cleared lysate was loaded onto a TALON metal-affinity resin column from BD Biosciences at 4ºC. 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 ß-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 ß-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 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 ß-mercaptoethanol) inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF) and lysed using Microfluidizer. The lysate was cleared by centrifugation.
Concentration:31 mg/mL
Ligand
MassSpec:
Crystallization: Purified UBC E2E2 was crystallized using the sitting drop vapor diffusion method. Crystals grew when the protein (20 mg/mL) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop was equilibrated against a reservoir solution containing 25% PEG 3350, 0.1M bis-Tris, pH 5.5.
NMR Spectroscopy:
Data Collection:
Data Processing: