UBE2J2

PDB:2F4W

Revision

Revision Type:created
Revised by:created
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Entry Clone Accession:gi:18490186
Entry Clone Source:MGC
SGC Clone Accession: Ubc57.001.185 plate SDC045:H4
Tag:N-terminal His-tag with integrated thrombin-cleavage site MGSSHHHHHHSSGLVPRGS.
Host:E.coli BL21 (DE3)

Construct

Prelude:
Sequence:

GSMSSTSSKRAPTTATQRLKQDYLRIKKDPVPYICAEPLPSNILEWHYVVRGPEMTPYEGGYYHGKLIFPREFPFKPPSIYMITPNG
RFKCNTRLCLSITDFHPDTWNPAWSVSTILTGLLSFMVEKGPTLGSIETSDFTKRQLAVQSLAFNLKDKVFCELFPEVVEEIKQKQK
AQDELSSRPQTLP

Vector:p28a-LIC

Growth

Medium:TB
Antibiotics:
Procedure:UBC E2J2 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 15degC. The culture was centrifuged and the cell pellets were collected and stored at -80degC.

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, wash buffer B and again wash buffer A and the protein was eluted with Elution Buffer. The protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with GF buffer,and concentrated by ultrafiltration.

Extraction

Procedure
The cell pellet was resuspended in lysis buffer inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF) and lysed using Microfluidizer. The lysate was cleared by centrifugation.
Concentration:
Ligand
MassSpec:
Crystallization: Purified UBC E2J2 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: