UBE2E1

PDB:3BZH

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_997236
Entry Clone Source:ubc47.NM_003341.EXT.janosch_ube2e1.pETkan
SGC Clone Accession:ubc47.001.193.129A11 (SDC129A11)
Tag:N-terminal: MHHHHHHSSGRENLYFQ*G (removed after metal-affinity chromatography)
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

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mhhhhhhssgrenlyfq*gMSDDDSRASTSSSSSSSNQQTEKETNTPKKKESKVSMSKNSKLLSTSAKRIQKELADITLDPPPNCS
AGPKGDNIYEWRSTILGPPGSVYEGGVFFLDITFTPEYPFKPPKVTFRTRIYHCNINSQGVICLDILKDNWSPALTISKVLLSICSL
LTDCNPADPLVGSIATQYMTNRAEHDRMARQWTKRYAT
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Vector:pET28-MHL

Growth

Medium:TB
Antibiotics:
Procedure: The protein was expressed in E. coli BL21 (DE3) grown in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin at 37ºC to an OD600 of 7.5. Protein expression was induced 0.05 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15ºC. The culture was centrifuged (12,000 x g, 15 minutes) and cell pellets collected and stored at -80ºC.

Purification

Procedure
The cleared lysate was loaded onto a 3 mL TALON metal-affinity resin column (BD Biosciences) at 4ºC. The column was washed with 10 mL Wash buffer A, 10 mL Wash buffer B, and 10 mL Wash buffer A. The protein was eluted with 6 mL Elution buffer.

His-Tag was removed by incubation with TEV protease (10 μg/ mg protein) overnight at 4°C.

The protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with Gel Filtration buffer. Fractions containing protein (analyzed by SDS-PAGE) were pooled and concentrated by ultrafiltration using an Amicon Ultra centrifugal filter with 10 kD cutoff to a final concentration of 30 mg/ml.

Protein yield was 4.5 mg per liter bacterial culture.

Extraction

Procedure
The cell pellet from a 2 L culture was resuspended in 50 ml Lysis buffer, lysed using a Microfluidizer at 18,000 PSI, and cleared by centrifugation at 40,000 x g for 30 min.
Concentration:15 mg/ml
Ligand
MassSpec: Mass-spectroscopy by LCMS showed that the product was pure and of correct molecular weight
Crystallization: Crystals of the UBE2E1 were grown at 298 K using the hanging drop method by mixing 1 volume of 15 mg/ml protein with 1 volume of well solution consisting of 2.2 M ammonium sulfate, 0.1 M bis-Tris-propane, pH 9.0 and 1 mM dithiothreitol. The crystals were cryoprotected by Paratone N/paraffin oil mixture (1:1).
NMR Spectroscopy:
Data Collection:
Data Processing: