UBE2Q1

PDB:2QGX

Revision

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Tag:mgsshhhhhhssglvpr*gs
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvpr*GSGSVQVTDRLMKELRDIYRSQSFKGGNYAVELVNDSLYDWNVKLLKVDQDSALHNDLQILKEKEGADF
ILLNFSFKDNFPFDPPFVRVVSPVLSGGYVLGGGAICMELLTKQGWSSAYSIESVIMQISATLVKGKARVQFGANKSQYSLTRAQQS
YKSLVQIHEKNGW

Vector:p28a-LIC-Thrombin

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 then induced with 0.05 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15ºC. The culture was centrifuged (12,000 x g, 15 min) and cell pellets collected and stored at -80ºC.

Purification

Procedure
Column 1: TALON metal-affinity resin column (BD Biosciences)
Column 2: HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham)

The cleared lysate from a 2 liter culture was loaded onto 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 then 30 ml Wash buffer A, and the protein was eluted with 6 ml Elution buffer.

His-tags was removed by incubation of the protein with 1 U thrombin per mg protein (overnight at 4 °C), and the protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with Gel Filtration buffer and concentrated by ultrafiltration using Amicon Ultra centrifugal filter with 10kD cutoff.

Extraction

Procedure
The cell pellet from a 2 liter culture was resuspended in 50 ml Lysis buffer and lysed using Microfluidizer at 18,000 psi. The lysate was cleared by centrifugation (40,000 x g, 30 minutes).
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were grown in hanging drops by mixing 2 microL protein solution (10 mg/ml) with 2 microL well solution (22% PEG4000, 0.1 M Tris-Cl, pH7.5, 0.12 M $MgCl_2$, 1 mM DTT) at 21°C. For cryoprotection, the crystals were soaked in well solution supplemented with 20% glycerol.
NMR Spectroscopy:
Data Collection:
Data Processing: