UBE2H

PDB:1YH6

Revision

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Entry Clone Accession:gi 33873494
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SGC Clone Accession:ubc40.001.160; plate SDC005:D10
Tag:N-terminal His-tag with integrated thrombin protease site: mgsshhhhhhssglvprgs.
Host:E.coli BL21-Gold (DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsMSSPSPGKRRMDTDVVKLIESKHEVTILGGLNEFVVKFYGPQGTPYEGGVWKVRVDLPDKYPFKSPSI
GFMNKIFHPNIDEASGTVCLDVINQTWTALYDLTNIFESFLPQLLAYPNPIDPLNGDAAAMYLHRPEEYKQKIKEYIQKYATEEALK
EQEEG

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure:UBE2H was expressed in E. coli BL21-Gold (DE3) in Terrific Broth (TB) in the presence of 50 µg/mL of kanamycin. The culture was incubated at 37 ºC to an OD600 of 4, cooled to 20 ºC, and then induced with 1 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 20 ºC. For growth in selenomethionine, BL21-Gold (DE3) was cultured in M9 medium at 37 ºC to an OD600 of 0.8, cooled to 20 ºC, and then induced with 1 mM IPTG overnight at 20 ºC.

Purification

Procedure
The flow-through of the DE52 column was loaded onto a Ni-NTA Superflow (Qiagen) column at 4 ºC. The column was washed with wash buffer (50 mM HEPES, pH7.5, 0.5 M NaCl, 5% glycerol, and 40 mM imidazole), and the protein was eluted with elution buffer (50 mM HEPES, pH7.5, 0.5 M NaCl, 5% glycerol, and 250 mM imidazole). The purified protein was dialyzed overnight against crystallization buffer (10 mM HEPES, pH7.5, 0.5 M NaCl) with 1 mM b-mercaptoethanol at 4 ºC and concentrated using Amicon Ultra centrifugal filter devices (Millipore).

Extraction

Procedure
The culture was harvested and the cell pellet suspended in binding buffer (50 mM HEPES, pH 7.5, 0.5 M NaCl, 5% glycerol, and 5 mM imidazole) with protease inhibitor (1 mM benzamidine-HCl and 1 mM phenylmethyl sulfonyl fluoride, PMSF) and kept at - 80 ºC. Before purification, the cell pellet was thawed overnight at 4 ºC. The cells were lysed by a combination of 0.5% CHAPS (Pierce) and microfluidization. Lysate was cleared by centrifugation and passed through a DE52 (Whatman) column.
Concentration:15 mg/mL
Ligand
MassSpec:
Crystallization: Native purified human UBE2H was crystallized using the sitting drop vapor diffusion method. One mL of the diluted protein solution (5 mg/mL) was mixed with 1 mL of the reservoir solution consisting of 25% PEG3350, 0.2 N NaCl, and 0.1 M bis-Tris, pH 5.5. Crystals appeared overnight.
NMR Spectroscopy:
Data Collection:
Data Processing: