Human UBCE2I + HIP2 complex

PDB:2O25

Revision

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Entry Clone Accession:gi21536483 (HIP2); gi42659538 (ubc9)
Entry Clone Source:MGC
SGC Clone Accession:UBE2I: ubc32.001.158.53F03
HIP2: ubc45.001.200.06B08
Tag:N-Terminal His-tag with integrated thrombin-cleavage site:
mgsshhhhhhssggvprGS
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

UBCE2I:

mgsshhhhhhssggvprGSMSGIALSRLAQERKAWRKDHPFGFVAVPTKNPDGTMNLMNWEC
AIPGKKGTPWEGGLFKLRMLFKDDYPSSPPKCKFEPPLFHPNVYPSGTVCLSILEEDKDW
RPAITIKQILLGIQELLNEPNIQDPAQAEAYTIYCQNRVEYEKRVRAQAKKFAPS
HIP2:
mgsshhhhhhssggvprGSMANIAVQRIKREFKEVLKSEETSKNQIKVDLVDENFTELRGEIAGP
PDTPYEGGRYQLEIKIPETYPFNPPKVRFITKIWHPNISSVTGAICLDILKDQWAAAMTLRT
VLLSLQALLAAAEPDDPQDAVVANQYKQNPEMFKQTARLWAHVYAGAPVSSPEYTKKIE
NLCAMGFDRNAVIVALSSKSWDVETATELLLSN
Vector:p28a-LIC-thrombin

Growth

Medium:TB
Antibiotics:

Procedure: The proteins were individually expressed in E. coli BL21 (DE3) grown in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin at 37 degC to an OD_{600} of 7.5. Protein expression was then induced with isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.05 mM, and incubated overnight at 15 degC. The culture was centrifuged and the cell pellets were collected and stored at -80 degC.

Purification

Procedure
UBE2I and HIP2 were purified as individual proteins as follows. The cleared lysate was loaded onto a TALON metal-affinity resin column (BD Biosciences) at 4 degC (1.5 ml settled gel volume per liter original cell culture). The column was washed with 10 ml wash buffer A, 10 mlwash buffer B and then with 30 ml wash buffer A, and the protein was eluted with 6 ml elution buffer. His-tags were removed by incubation of the proteins with thrombin, and they were combined in an equimolar ration and further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with GF buffer and concentrated by ultrafiltration to a final protein concentration of 26 mg/ml using Amicon Ultra centrifugal filter with 10kD cutoff.

Extraction

Procedure
The cell pellet was resuspended in lysis buffer containing protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF) and lysed using Microfluidizer. The lysate was cleared by centrifugation.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were grown in hanging drops by mixing 2 microL protein solution with 2 microL well solution (16% PEGMME 5000, 0.1 M bis-Tris-HCl, pH 6.0, 1 mM DTT) at 21 degC. For cryoprotection, the crystals were soaked in well solution supplemented with 20% ethylene glycol.
NMR Spectroscopy:
Data Collection:
Data Processing: