HIP2 (E2-25)

PDB:1YLA

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi 30048149
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal His-tag with integrated thrombin-cleavage site MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21 (DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsMANIAVQRIKREFKEVLKSEETSKNQIKVDLVDENFTELRGEIAGPPDTPYEGGRYQLEIKIPETYPF
NPPKVRFITKIWHPNISSVTGAICLDILKDQWAAAMTLRTVLLSLQALLAAAEPDDPQDAVVANQYKQNPEMFKQTARLWAHVYAGA
PVSSPEYTKKIENLCAMGFDRNAVIVALSSKSWDVETATELLLSN

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure:UBC E2-25K 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. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.05 mM, and incubated overnight at 15ºC. The culture was centrifuged and the cell pellets were collected and stored at -80ºC.

Purification

Procedure
The cleared lysate was loaded onto a TALON metal-affinity resin column from BD Biosciences at 4ºC. The column was washed with wash buffer A (10mM Tris-HCl pH 8.0, 0.5 M NaCl, 5% glycerol, 10 mM imidazole, 1 mM ß-mercaptoethanol), wash buffer B (same as wash buffer A but containing 0.05% Tween with protease 20) and again wash buffer A, and the protein was eluted with 10 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 200 mM imidazole, 1 mM ß-mercaptoethanol. The protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with 20 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 2mM dithiothreitol and concentrated by ultrafiltration.

Extraction

Procedure
The cell pellet was resuspended in lysis buffer (10 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 5% glycerol, 2 mM imidazole, 1 mM ß-mercaptoethanol) inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF) and lysed using Microfluidizer. The lysate was cleared by centrifugation.
Concentration:
Ligand
MassSpec:
Crystallization: Purified UBC E2-25K was crystallized using the hanging 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 18% PEG 8000, 0.1M calcium acetate, 0.1M sodium cacodylate, pH 6.6.
NMR Spectroscopy:
Data Collection:
Data Processing: