UHRF1

PDB:3FL2

Revision

Revision Type:created
Revised by:created
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Entry Clone Accession:NP_037414
Entry Clone Source:ubh12.BC113875.OBS.MHS4426-98361361.pCR-BluntIITOPO
SGC Clone Accession:ubh12.672.793.133H12 (SDC133H12)
Tag:N-terminal: MGSSHHHHHHSSGLVPRGS
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsEPYSLTAQQSSLIREDKSNAKLWNEVLASLKDRPASGSPFQLFLSKVEETFQCICCQELVFRPITTVC
QHNVCKDCLDRSFRAQVFSCPACRYDLGRSYAMQVNQPLQTVLNQLFPGYGNGR

Vector:pET28a-LIC

Growth

Medium:
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 37 °C to an OD600 of approx. 7. Protein expression was induced with 0.1 mM isopropyl-1-thio-D-galactopyranoside overnight at 15 °C. The culture was centrifuged (12,000 x g, 15 minutes) and cell pellet was collected and stored at -80 °C.

Purification

Procedure
The cleared lysate was loaded onto a 3 mL TALON metal-affinity resin (Clontech) column 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. The His-tag was removed by overnight incubation of the protein with thrombin at 4ºC.

The protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare) equilibrated with Gel Filtration buffer. Fractions containing protein were pooled and concentrated by ultrafiltration to a final protein concentration of 120 mg/ml.

The yield of the protein was 4 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 p.s.i., and cleared by centrifugation at 40,000 x g for 30 min.
Concentration:
Ligand
MassSpec: Mass-spectroscopy by LC/MS showed pure product of correct molecular weight corresponding RING domain with N-terminal addition of glycine and serine residues from the tag.
Crystallization: Crystals of the UHRF1 RING domain were grown at 293K using the hanging drop method by mixing 1 volume of protein solution (60 mg/ml in 20 mM HEPES, pH 7.0, 300 mM NaCl, 1 mM TCEP) with 1 volume of well solution consisting of 27% PEG MME 2000, 0.1 M Tris-HCl, pH 8.5. The crystals were cryoprotected by immersion in Paratone N diluted (1 : 1) with paraffin oil.
NMR Spectroscopy:
Data Collection:
Data Processing: