UHRF1

PDB:3CLZ

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:ubh12.414.617.125B05 (SDC125B05)
Tag:N-terminal: M
C-terminal: AHHHHHH
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

mPSNHYGPIPGIPVGTMWRFRVQVSESGVHRPHVAGIHGRSNDGAYSLVLAGGYEDDVDHGNFFTYTGSGGRDLSGNKRTAEQSCDQ
KLTNTNRALALNCFAPINDQEGAEAKDWRSGKPVRVVRNVKGGKNSKYAPAEGNRYDGIYKVVKYWPEKGKSGFLVWRYLLRRDDDE
PGPWTKEGKDRIKKLGLTMQYPEGYLEALANahhhhhh

Vector:pNIC-CH

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

Purification

Procedure
The cleared lysate was loaded onto a 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 10 mL Wash buffer A. The protein was eluted with 6 mL Elution buffer.

The protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with Gel Filtration buffer. Fractions containing protein (analyzed by SDS-PAGE) were pooled and concentrated by ultrafiltration using an Amicon Ultra centrifugal filter with 10 kD cutoff to a final concentration of 34 mg/ml.

The yield of the protein was 4 mg per liter of 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 PSI, and cleared by centrifugation at 40,000 x g for 30 min.
Concentration:34 mg/ml
Ligand
MassSpec: Mass-spectroscopy by LC/MS showed pure product of correct molecular weight corresponding the UHRF1 SRA domain.
Crystallization: The protein at 10 mg/ml was mixed, in a molar ratio of 1:1.5, with a double-stranded DNA obtained by annealing of two oligonucleotides, 5Â□-GGGCCXGCAGGG (X = 5-methylcytosine) and 5Â□-CCCTGCGGGCCC synthesized by Oligos Etc., and incubated for 1 h on ice. Crystals of the complex were grown at 298 K using the hanging drop method by mixing 1 volume of the complex solution with 1 volume of well solution, consisting of 12% PEG1500, 0.2 M NaCl, 1 mM TCEP, 0.1 M bis-Tris, pH 7, and 0.4 volume of 30% xylitol. The crystals were cryoprotected by immersion in the well solution mixed in 1:1 ratio with a water solution containing 20% (w/v) sucrose, 4% (w/v) glucose, 18% (v/v) glycerol and 18% (v/v) ethylene glycol.
NMR Spectroscopy:
Data Collection:
Data Processing: