

UHRF1

PDB:3DB4

Revision

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Entry Clone Accession:NP_037414

Entry Clone Source:ubh12.BC113875.OBS.MHS4426-98361361.pCR-BluntIITopo

SGC Clone Accession:ubh12.126.285.130H02 (SDC130H02)

Tag:N-terminal: MHHHHHHSSGRENLYFQG

Host:BL21 (DE3)

Construct

Prelude:

Sequence:

mhahhhhhssgrenlyfqgMWDETELGLYKVNEYVDARDTNMGAWFEAQVVRVTRKAPSREPCSSTSRALEEDVIYHVKYDDYPEN
GVVQMNSRDRVRARARTIIKWQDLEVGQVVMLNYNPDPNPKERGFWYDAEISRKRETRTARELYANVVLGDDSLNDCRIIFVDEVFKIE
RPGE

Vector:pET28-MHL

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 7.5. Protein expression was induced 0.05 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15°C. The culture was centrifuged (12,000 x g, 15 minutes) and cell pellets collected and stored at -80°C. In order to obtain the selenomethionyl derivative of the UHRF1 SRA domain, the cells were grown in M9 medium supplemented with glycerol using a M9 SeMET High-Yield growth media kit package (MD045004-50L, Medicilon) according to manufacturer's instructuion and the protein was purified as below.

Purification

Procedure

The cleared lysate was loaded onto a 3 mL TALON metal-affinity resin column (BD Biosciences) at 4°C. 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 N-terminal His-tag was removed by overnight incubation of the protein with TEV protease at 4 C.

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.

Final purification was achieved by ion-exchange chromatography on a 5-ml HiTrapQ column using linear 0 - 50 % gradient of Ion-exchange buffer B in Ion-exchange buffer A. The target protein eluted around 30% buffer B. The corresponding fractions were combined and protein was concentrated by ultrafiltration using an Amicon Ultra centrifugal filter with 10 kD cutoff to a final concentration of 28 mg/ml.

The yields of the protein and its selenomethionyl derivatives were approx. 7 and 9 mg per liter of bacterial culture, respectively.

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:

Ligand

MassSpec: Mass-spectroscopy by LC/MS showed pure product of correct molecular weight corresponding selenomethionyl derivative of the tandem-Tudor domain with addition of an N-terminal glycine residue from the tag and all four methionine residues substituted with selenomethionine.

Crystallization: Crystals of the UHRF1 tandem-Tudor domain selenomethionyl derivative were grown at 298 K using the hanging drop method by mixing 1 volume of 23 mg/ml protein with 1 volume of well solution consisting of 10% PEG 8000, 0.2 M ammonium sulfate, 0.1 M sodium cacodylate, pH 6.5, and 1 mM TCEP. 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: