UHRF2

PDB:4TVR

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:SGC:33-H4
Entry Clone Source:SGC cDNA collection
SGC Clone Accession:YTC015-B01
Tag:N-terminal His6-tag, removed
Host:BL21(DE3)-V2R-pRARE2

Construct

Prelude:UHRF2:N109.D395-Del167-174.E383AThis constructs contains both deletion and mutation.
Sequence:

gNQPSTSARARLIDPGFGIYKVNELVDARDVGLGAWFEAHIHSVTRASDGQSRGKTPLKNGNIKHKSKENTNKLDSVPSTSNSDCVA
ADEDVIYHIQYDEYPESGTLEMNVKDLRPRARTILKWNELNVGDVVMVNYNVESPGQRGFWFDAEITTLKTISRTKKELRVKIFLGG
SEGTLNDCKIISVDEIFKIERPGAHPLSFADGKFLRRNDPECDLCGGDPEKKCHSCSCRVCGGKHEPNMQLLCDECNVAYHIYCLNP
PLDKVPAEEYWYCPSCKTD

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:LEX Bubbling. The target protein was expressed in E. coli by inoculating 30 mLof overnight culture grown in Luria-Bertani medium into a 2 L of TerrificBroth medium in the presence of 50 ug/mL kanamycin and 34 ug/mLchloramphenicol at 37 degree. When the OD600 of the culture reached ~1.5, the temperature was lowered to 18 degree and theculture was induced with 0.25 mM IPTG. The cells were allowed to growovernight before harvested by centrifugation

(7,000 rpm Beckman JLA-8.1000rotor 12min) and flash frozen in liquid nitrogen and stored at -80 degree.

Purification

Procedure
The lysate was clarified by centrifugation at 16,000 rpm (25,800xgRCF(average)) for 60 minutes. 6mL Ni-NTA beads (50% slurry) were then added intothe supernatant and the mixture were put on rotary drum for 60 minutues forinding before passing onto a Bio-rad open gravity column. The beads in theopen coumn was then washed with 50 mL lysis buffer followed by with 15 mL washing buffer. Bound proteins were eluted using 15 mL elution buffer. The N-terminalHis-tag was reomoved by overnight incubation with TEV protease (1:30 w/w) at 4degree during dialysis against the dialysis buffer. Uncut proteins and TEVprotease were removed by passing the solution through 3mL Ni-NTA beads, andthe target protein was further purified by anion-exchange chromatography on 5mL HiTrap Q column(GE Healthcare). Then the protein was further purified bygel filtration on a HighLoad 16/60 Superdex 75 column (GE Healthcare)preequilibrated with gel filtration buffer. Fractions containing targetprotein were pooled and concentrated by centrifugal filters (Amicon mwco10kDa). The final yield of the protein was about 10 mg per litre bacterialculture and the purity is above 97% judging from SDS-PAGE.

Extraction

Procedure
2L cell pellet was resuspended in a total volume of 200 ml lysis buffer with1mM PMSF/Benzamidine freshly added and the cells disrupted by sonication for 1 mins at 5" on 7" off duty cycle at 120W output power,
Concentration: Concentration used for crystallization 23.1 mg/mL
Ligand
MassSpec: Protein expected 31315.3 g/mol, measured 31315.5 g/mol.
Crystallization: Crystal of UHRF2 used for structure determination was harvested from initialscreen plate (SGC-II screens). It was grown at 298K using the sitting drop method by mixing0.5 uL of protein with 0.5 uL well solution consisting of 25% PEG-3350, 0.2 MNaCl, 0.1 M HEPES pH 7.5, 5% glycerol. The crystal was cryoprotected firstlyby immersion into a cryo containing 12% glycerol in well solution, thenimmersion in Paratone.
NMR Spectroscopy:
Data Collection:
Data Processing: