UHRF2

PDB:3OLN

Revision

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Entry Clone Accession:uhrf2.NM_152896.ORI.RC219579.pCMV6
Entry Clone Source: Origene
SGC Clone Accession:ubh14.0420-0648.195E09 (SDC195E09)
Tag:N-terminal tag: MGSSHHHHHHSSGLVPRGS
Host: Competent BL21 (DE3) cells

Construct

Prelude:
Sequence:

MGSSHHHHHHSSGLVPRGSTESRRDWGRGMACVGRTRECTIVPSNHYGPIPGIPVGSTWRFRVQVSEAGVHRPHVGGIHGRSNDGAY
SLVLAGGFADEVDRGDEFTYTGSGGKNLAGNKRIGAPSADQTLTNMNRALALNCDAPLDDKIGAESRNWRAGKPVRVIRSFKGRKIS
KYAPEEGNRYDGIYKVVKYWPEISSSHGFLVWRYLLRRDDVEPAPWTSEGIERSRRLCLRLQYPAGYPSDKEGK

Vector:pET28a-LIC vector (GenBank accession EF442785)

Growth

Medium:TB (Sigma T0918) supplemented with 150 mM glycerol, 100 µM Kanamycin and 600 µl antifoam 204 (Sigma A-8311)
Antibiotics:
Procedure: Competent BL21 (DE3) cells (Invitrogen C6000-03) were transformed and grown using the LEX system (Harbinger BEC) at 37 oC in 2L bottles (VWR 89000-242) containing 1800 ml of growth medium. When OD_{600} ~ 6 was reached, the temperature was reduced to 15 oC. One hour later protein expression was induced with 100 µM IPTG (BioShop IPT001), and the culture was incubated overnight (16 hours) at 15 oC. Cell pellets were collected by centrifugation (12,227 x g, 20 min), frozen and stored at -80 oC.

Purification

Procedure
After resuspension in 30 mL per litre bacterial culture of Lysis Buffer, cells were lysed using a Microfluidics M110-EH microfluidizer at 18,000 psi. The cleared lysate was loaded onto a 3 mL TALON metal-affinity resin column (BD Biosciences) at 4ºC. The column was washed 3 times with 15 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) using Gel Filtration Buffer. Fractions containing protein corresponding to the UHRF2 peak were pooled and concentrated by ultrafiltration. The N-terminal His-tag was removed by overnight incubation with thrombin at 4°C, and the protein was further purified by cation-exchange chromatography on 5-ml HiTrap SP column equilibrated with Cation-Exchange Chromatography Buffer A using 0-50% linear gradient formed by mixing Cation-Exchange Buffer A with Cation-Exchange Chromatography Buffer B. The final yield of the protein was 40 mg per litre bacterial culture.

Extraction

Procedure
After resuspension in 30 mL per litre bacterial culture of Lysis Buffer, cells were lysed using a Microfluidics M110-EH microfluidizer at 18,000 psi.
Concentration:
Ligand
MassSpec:MW = 24,477.7 g/mol
Crystallization: Crystals of the UHRF2 SRA domain were grown at 291 K using the hanging drop method by mixing equal volumes of the protein solution (10 mg/ml) and Crystallization Buffer 1 (19 % PEG3350, 0.1 M HEPES-Na, pH 7.0, 5% MPD, 0.2 M NaCl, 1 mM DTT). Crystals (plate clusters) grew in 3-7 days and were further used for microseeding as follows: a hanging drop was prepared by mixing 1.5 ul protein solution at 7 mg/ml with 1.5 ul Crystallization Buffer 2 (23% PEG3350, 0.1 M HEPES-Na, pH 7.0, 5% MPD, 0.2 M NaCl, 1 mM DTT) and 0.3 ul microseed suspension, and equilibrated against 300 ul Crystallization Buffer 2 in the well for 5-7 days. The obtained single crystals were cryoprotected by immersion in the Crystallization Buffer 2 mixed, in a 1 : 1.2 (v/v) ratio, with a cryoprotecting mixture that consisted of 20% (w/v) sucrose, 4% (w/v) glucose, 18% (v/v) glycerol and 18% (v/v) ethylene glycol in water, and placed in liquid nitrogen.
NMR Spectroscopy:
Data Collection:
Data Processing: