UHRF1

PDB:4QQD

Revision

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Entry Clone Accession:NP_001041666.1.
Entry Clone Source: Open Biosystems
SGC Clone Accession:UHRF1_18 (SDC130:H02): M139-E298
Tag:N-terminal tag: MHHHHHHSSGRENLYFQG
Host: Escherichiacoli BL21 (DE3)

Construct

Prelude:
Sequence:

MHHHHHHSSGRENLYFQGMWDETELGLYKVNEYVDARDTNMGAWFEAQVVRVTRKAPSRDEPCSSTSRPALEEDVIYHVKYDDYPEN
GVVQMNSRDVRARARTIIKWQDLEVGQVVMLNYNPDNPKERGFWYDAEISRKRETRTARELYANVVLGDDSLNDCRIIFVDEVFKIE
RPGE

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure: Lex system, 37 °C 3-4h, 15 °C 18-24h

Purification

Procedure
IMAC: Unclarified lysate was mixed with 3-4 mL of Ni-NTA superflow Resin (Qiagen) per 200 mL lysate. The mixture was incubated with mixing for at least 30 minutes at 4°C. The mixture was then loaded onto an empty comLum (BioRad) and washed with 40 mL wash buffer. Samples were eluted from the resin by exposure to 2-3 column volumes (approx. 10-15 mL) of elution buffer. Concentration of eluted protein was estimated by OD280

Gel filtration chromatography: An XK 16x60 column (GE Healthcare) packed with HighLoad Superdex 75 resin (GE Healthcare) was pre-equilibrated with gel filtration buffer for 1.5 column volumes using an AKTA explorer (GE Healthcare) at a flow rate of 1.0 mL/min. The concentrate sample (approx. 3 mL, concentrated by using 15 mL concentrators with a 3,000 molecular weight cut-off (Amicon Ultra-15, UFC900524, Millipore) at 3750 rpm) from the IMAC step was loaded

onto the column at 1 mL/min, and 2mL fractions were collected into 96-well plates (VWR 40002-012) using peak fractionation protocols. Fractions observed by a UV absorption chromatogram to contain the protein were pooled.

Extraction

Procedure
Frozen cell pellet contained in bags (Beckman 369256) obtained from 2L of culture were thawed by soaking in warm water. Each cell pellet was resuspended in 200 mL lysis buffer and homogenized using an Ultra-Turrax T8 homogenizer (IKA Works) at maximal setting for 30-60 seconds per pellet. Cell lysis was accomplished by sonication (Virtis408912, Virsonic) on ice: the sonication protocol was 10 seconds pulse at 80% of maximal frequency (8.0), 10 seconds rest, for 10 minutes total sonication time per pellet.
Concentration: Purified proteins were concentrated using 15 mL concentrators with a 5,000 molecular weight cut-off (Amicon Ultra-15, UFC900524, Millipore) at 3750 rpm, typically resulting in a final concentration around 20 mg/mL.
Ligand
inhibitor**MassSpec:**
Crystallization: Recombinant human UHRF1 tandem tudor domain was mixed with small organic molecule at molar ratio of 1:3 and co-crystallized using the sitting drop vapour diffusion method at 18 °C. The crystals were obtained in a buffer containing 20% PEG 3350, 0.2M MgNO4. Crystals were soaked in a cryoprotectant consisting of 100% reservoir solution and 15% glycerol.
NMR Spectroscopy:
Data Collection:
Data Processing: