JMJD1B

PDB:4C8D

Revision

Revision Type: created
Revised by: created
Revision Date: created
Entry Clone Accession: NM_016604
Entry Clone Source:
SGC Clone Accession:
Tag: C-terminal, TEV cleavable hexahistidine tag
Host:

Construct
Prelude:

Sequence:
MTSHSWLCDGRLLCLHDPSNKNNWKIFRECWKQGQPVLVSGVHKKLKSELWKPEAFSQ
EFGDQDVDLVNCRNCAIISDVKVRDFWDGFEIICKRLRSEDGQPMVLKLKDWPPGEDFR
DMMPTRFEDLMENLPLPEYTKRDGRLNLASRLPSYFVRPDLGPKMYNAYGLITAEDRRV
GTTNLHLDVSDAVNVMVYVGIPIGEGAHDEEVLKTIDEGDADEVTKQRIHDGKEKPGAL
WHIYAAKDAEKIRELLRKVGEEQGQENPPDHDPIHDQSWYLDQTLRKRLYEEYGVQGW
AIVQFLGDAVFIPAGAPHQVHNLYSCIKVAEDFVSPEHVKHCFRLTQEFAGNLYFQ

Note: a change from E to G within the TEV protease site does not affect cleavage.

Vector:pNIC-CTHF

Growth

Medium:

Antibiotics:

Procedure:

Purification

Buffers

Procedure

Lysis/ Binding Buffer: 50mM HEPES pH 7.4, 500mM NaCl, 5% glycerol, 10 mM Imidazole pH 7, 0.5 mM TCEP, mixture of proteinase inhibitors.

Wash Buffer : 50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 40 mM Imidazole pH 7.4, 0.5mM TCEP

Elution Buffer:50 mM Hepes pH 7.4, 500 mM NaCl, 5% Glycerol, 250 mM Imidazole pH 7.4, 0.5mM TCEP

Gel Filtration buffer: 20 mM Hepes pH 7.4, 500 mM NaC, 5% glycerol, 0.5mM TCEP

Cell Lysis: The cells from one litre culture, were lysed by sonication. Cell debris and DNA were spun down at 56000x g, 45 min (Beckman JA 50.50 21500 rpm). The supernatant was collected to which Benzonase was added (1ul/litre culture).

Affinity binding to Ni-sepharose resin: The clarified cell extract was first batch bound to 2ml resin /litre culture. The slurry was rotated for 90 minutes at 4°C, then spun 15min/500xg/ 4°C.The supernatant was removed. The pelleted resin was loaded onto a glass column. The column was washed with 30CV of binding buffer, 5CV of wash buffer, and then eluted in aliquots with elution buffer.

TEV digestion: TEV was added to the eluted protein at a molar ratio of 1:20. The digestion was continued over night at 4C. The complete digestion was confirmed by mas spec.

Gel filtration, Hiload 16/60 Superdex S200 prep grade, 120 ml The digested protein was loaded on a gel filtration column, pre-equilibrated in GF buffer, at 1.2 ml/min. Eluted proteins were collected in 1.75 ml fractions and analyzed on SDS-PAGE.

Rebinding of the gel filtration pool: The pool was loaded onto a column with 0.4 ml Ni-sepharose, collecting the flow through and a gel filtration buffer wash.

Concentrating: The flow through was concentrated using a centricon centrifugal device with a 30kDa MWCO.The concentrated protein was centrifuged 10 min/ 14000rpm/4C. The final concentration, in the supernatant, was 24.1 mg/ml and yield 20 mg/litre culture. The protein was flash frozen and stored at -80°C in 70ml aliquots.

Extraction

Buffers

Procedure

Expression strain: BL-21(DE3)-R3-Rosetta (A homemade phage resistant version of BL21(DE3) containing the pRARE2 plasmid from Rosetta II (DE3) cells).

Transformation: The construct DNA was transformed into competent cells of the expression strain by a standard heat shock procedure.

Glycerol stock preparation: A number of colonies were used to inoculate 1 ml of LB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was incubated in a 37°C shaker overnight. The next day glycerol stocks were prepared from this overnight culture.

Expression: 10 ml of a glycerol stock was used to inoculate 120 ml of LB containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol, which was incubated at 37°C overnight. 10ml starter culture was used per litre TB, containing 50 µg/ml kanamycin. The culture was incubated at 37°C until OD~1.5, when the temperature of the incubator was reduced to 18°C. At 18°C, expression was induced with 0.1 mM IPTG and the culture continued o/n.

Cell harvest: Cells were pelleted at 6238x g for 15 min at 4°C, resuspended in lysis buffer (50mM HEPES pH7.4,10mM imidazole, 0.5M NaCl, 5% glycerol, 0.5mM TCEP and proteinase inhibitors), flash frozen in liquid nitrogen and stored at -80°C.

Concentration:

Ligand

MassSpec:Mass spec. characterisation: Measured: 40092.75 and 40224.10 Expected: 40294.8

Crystallization:0.50mM JMJD1BA-p056 was incubated with 2mM NOG and 6mM MnCl2 for 2.5 hrs, on ice. The protein was spun 10min/21000xg and the supernatant was used for setting up crystallization plates. The crystal was found in precipitant [0.1M BisTris buffer pH=5.5, 0.1M ammonium acetate, 27% PEG3350]. The cryo contained 25% glycerol and 75% precipitant.

NMR Spectroscopy:

Data Collection: Data was collected to a resolution of 2.5 Å, at Diamond beamline.

Data Processing: