ME1

PDB:2AW5

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:ME1A-s001
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal His tag with TEV cleavage site
Host:E.coli BL21DE3 R3

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmQRGYLLTRNPHLNKDLAFTLEERQQLNIHGLLPPSFNSQEIQVLRVVKNFEHLNSDFDRYLLLM
DLQDRNEKLFYRVLTSDIEKFMPIVYTPTVGLACQQYSLVFRKPRGLFITIHDRGHIASVLNAWPEDVIKAIVVTDGERILGLGDLG
CNGMGIPVGKLALYTACGGMNPQECLPVILDVGTENEELLKDPLYIGLRQRRVRGSEYDDFLDEFMEAVSSKYGMNCLIQFEDFANV
NAFRLLNKYRNQYCTFNDDIQGTASVAVAGLLAALRITKNKLSDQTILFQGAGEAALGIAHLIVMALEKEGLPKEKAIKKIWLVDSK
GLIVKGRASLTQEKEKFAHEHEEMKNLEAIVQEIKPTALIGVAAIGGAFSEQILKDMAAFNERPIIFALSNPTSKAECSAEQCYKIT
KGRAIFASGSPFDPVTLPNGQTLYPGQGNNSYVFPGVALGVVACGLRQITDNIFLTTAEVIAQQVSDKHLEEGRLYPPLNTIRDVSL
KIAEKIVKDAYQEKTATVYPEPQNKEAFVRSQMYSTDYDQILPDCYSWPEEVQ

Vector:pNIC-BSA4

Growth

Medium:
Antibiotics:
Procedure: Cells were grown for 14 hrs at 37°C in 10 mL TB medium, supplemented with Kanamycin (50 µg/mL), and were inoculated into 1 l TB medium with the same antibiotic supplement. The culture was grown at 37°C with shaking at 250 rpm. At on OD of 0.5 cells were induced with 1 mM IPTG and the temperature was lowered to 25°C. Growth was continued for an additional 18 hrs until a final OD of 8-10. Cells were collected by centrifugation, and the cell pellet was stored at -20°C until further use.

Purification

Procedure
Column 1: 1 mL HisTrap FF.

Buffers: Affinity binding buffer: 10mM Imidazole, 300mM NaCl, 50mM pH8.0 KHPO4, 0.5mM TCEP; Affinity wash buffer: 50mM Imidazole, 300mM NaCl, 50mM pH8.0 KHPO4, 0.5mM TCEP; Affinity Elution Buffer: 250mM Imidazole, 300mM NaCl, 50mM pH8.0 KHPO4, 0.5mM TCEP.

Procedure: The supernatant from the last extraction step was loaded on a Akta Express system, consisting of IMAC and gel filtration as consecutive chromatography steps. Before sample loading, columns were equilibrated with the buffers indicated (HisTrap with Affinity binding buffer; gel filtration with Gel Filtration Buffer). Fractions were collected and analysed by mass spectrometry and SDS / PAGE . Peaks after gel filtration were collected, pooled and concentrated using Centricon membrane (30 kDa MW cutoff) c oncentrators.

Column 2 : Superdex S75

Buffers : Gel Filtration Buffer: 10mM pH7.4 Hepes, 500mM NaCl, 5% glycerol, 0.5mM TCEP.

Extraction

Procedure
Pellets were resuspended in approximately 3x volume of lysis buffer, and thawed at 37°C in a water bath. Cells were mechanically disrupted in a French Press, and further sonicated in 10 sec intervals for 2 mins. PEI was added to a final concentration of 0.15%, and DNA was precipitated on ice for 30 mins. The suspension was centrifuged for 17.000 rpm for 30 mins at 4°C, and the supernatant was filtered through a 0.2 um Serum Scrodisc filter. Lysis buffer: 10mM Imidazole, 300mM NaCl, 50mM pH8.0 KHPO4 , 0.5mM TCEP, 1x complete PI EDTA free tablet/50mL.
Concentration:
Ligand
MassSpec:
Crystallization: Column 1: 1 mL HisTrap FF.

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NMR Spectroscopy:
Data Collection:
Data Processing: