SEC14L3

PDB:4UYB

Revision

Revision Type:created
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Entry Clone Accession:BC101004 (IMAGE:40006377)
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal, TEV protease cleavable hexahistidine tag
Host:

Construct

Prelude:
Sequence:

MHHHHHHSSGVDLGTENLYFQSMSGRVGDLSPKQAETLAKFRENVQDVLPALPNPDDYFLLRWLRARNFDLQKSEALLRKYMEFRKT
MDIDHILDWQPPEVIQKYMPGGLCGYDRDGCPVWYDIIGPLDPKGLLFSVTKQDLLKTKMRDCERILHECDLQTERLGKKIETIVMI
FDCEGLGLKHFWKPLVEVYQEFFGLLEENYPETLKFMLIVKATKLFPVGYNLMKPFLSEDTRRKIIVLGNNWKEGLLKLISPEELPA
QFGGTLTDPDGNPKCLTKINYGGEIPKSMYVRDQVKTQYEHSVQINRGSSHQVEYEILFPGCVLRWQFSSDGADIGFGVFLKTKMGE
RQRAGEMTEVLPSQRYNAHMVPEDGNLTCSEAGVYVLRFDNTYSFVHAKKVSFTVEVLLPDEGMQKYDKELTPV

MHHHHHHSSGVDLGTENLYFQ*SM is the purification tag plus TEV protease recognition site *.
Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:

Purification

Buffers
Procedure
Buffers Used:
Binding/Lysis Buffer: 50 mM Hepes (pH 7.5), 500 mM NaCl, 5% Glycerol, 20 mM Imidazole pH 7.5, 0.5 mM TCEP
Wash Buffer: 50 mM Hepes (pH 7.5), 500 mM NaCl, 5% Glycerol, 40 mM Imidazole pH 7.5, 0.5mM TCEP
Elution Buffer: 50 mM Hepes (pH 7.5), 500 mM NaCl, 5% Glycerol, 250 mM Imidazole pH 7.5,

0.5mM TCEP
Gel Filtration Buffer: 10 mM Hepes (pH 7.5), 500 mM NaCl, 5% Glycerol, 0.5mM TCEP
Cell Lysis
Cell pellets were resuspended in 4 mL lysis buffer per gram of cell pellet and lysed by passing through the constant cell disruptor at a constant pressure of 15KPa. The cell debris were pelleted at 35 000 g and the supernatant used for further purification.

Column 1
Ni-NTA (2 mL volume in a gravity-flow column).
The clarified cell extract was incubated with 4 mL pre-equilibriated 50% Ni_NTA bead suspension for 1 hour at 4°C with rotation. The suspension was centrifuged at 900g for 10 min. The supernatant was poured away and the beads were resuspended in lysis buffer and transferred onto a gravity column. The column was then washed with 20ml Binding Buffer (2 x 10ml) and 20 ml Wash Buffer (2 x10 ml). The protein was eluted with 10 ml of elution Buffer in 5 x 2 ml fractions.

Column 2
Superdex s200 16/60 Gel Filtration.
Elution and wash fractions containing the target protein were concentrated to 5 mL and applied the size exclusion chromatography column, s200 (pre-equilibriated in GF buffer) at 1.0 ml/min. 1.0 ml fractions were collected.

Enzymatic treatment and purification
The N-terminal His6- tag was cleaved by incubating overnight with TEV at 4°C. Cleaved protein was purified by batch binding on 1ml pre-equilibriated 50% Ni-NTA bead solution. The column was then washed with 2x1ml Gel Filtration buffer,2x1ml Binding buffer, 2x1ml Wash buffer, and finally 2x1ml of Elution buffer.

Column 3
The protein was diluted 10 fold with buffer A (25mM Tris pH 8.5, 5% glycerol, 0.5mM TCEP) and loaded onto 1 mL HiTrapQ column. The protein was eluted in gradient to 1M NaCl.

Extraction

Buffers
Procedure
Expression strain
BL21(DE3)-R3-pRARE2

A glycerol stock was used to inoculate 60 ml of LB media containing 50ug/ml kanamycin and 50 ug/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day this starter culture was used to inoculate 6L of TB media (7 ml starter culture used per 1L) containing 50 ug/ml kanamycin. When the OD600 reached approximately 0.6 the temperature was reduced to 18°C and after a further 30 minutes the cells were induced by the addition of 0.1 mM IPTG. Expression was continued overnight.

Cell harvest
Cells were harvested by centrifugation at 6000g after which the media were poured out and the cell pellet either placed in a -80°C freezer or used directly for purification.
Concentration: To set up plates the sample was concentrated to 11.5 mg/ml using a 10 kDa mwco concentrator.
Ligand

MassSpec: Expected mass: 46149.4 Da
Measured mass: 46151.1 Da
Crystallization: Crystals were grown by vapour diffusion in sitting drop at 20°C. A sitting drop consisting of 100 nl protein and 50nl well solution was equilibrated against well solution containing 20% PEG6000, 0.8M lithium chloride and 0.1M citrate pH 4.2
NMR Spectroscopy:
Data Collection: Resolution: 1.45 Å
X-ray source: Diamond Light Source beamline IO3
Data Processing: