SEC14L4

PDB:4TLG

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession: BC139912
Entry Clone Source: GeneScript
SGC Clone Accession:
Tag:N-terminal, TEV protease cleavable hexahistidine tag
Host:

Construct

Prelude:
Sequence:

"MHHHHHHSSGVDLGTENLYFQSMSSRVGDLSPQQQEALARFRENLQDLLPILPNADDYFLLRWLRARNFDLQKSEDMLRRHMEFRK
QQDLDNIVTWQPPEVIQLYDSGGLCGYDYEGCPVYFNIIGSLDPKGLLLSASKQDMIRKRIKVCELLLHECELQTQKLGRKIEMALM
VFDMEGLSLKHLWKPAVEVYQQFFSILEANYPETLKNLIVIRAPKLFPVAFNLVKSFMSEETRRKIVILGDNWKQELTKFISPDQLP
VEFGGTMTDPDGNPKCLTKINYGGEVPKSYYLCEQVRLQYEHTRSVGRGSSLQVENEILFPGCVLRWQFASDGGDIGFGVFLKTKMG
EQQSAREMTEVLPSQRYNAHMVPEDGSLTCLQAGVYVLRFDNTYSRMHAKKLSYTVEVLLPDKASEETLQSLKAMRPSPTQ"

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, 0.01mM TCEP
Wash Buffer: 50 mM Hepes (pH 7.5), 500 mM NaCl, 5% Glycerol, 40 mM Imidazole pH 7.5, 0.01mM TCEP
Elution Buffer: 50 mM Hepes (pH 7.5), 500 mM NaCl, 5% Glycerol, 250 mM Imidazole pH 7.5,

0.01mM TCEP
Gel Filtration Buffer: 10 mM Hepes (pH 7.5), 500 mM NaCl, 5% Glycerol, 0.01mM TCEP

Cell Lysis
Cell pellets were dissolved in approximately 50ml lysis buffer and broken by passing through the homogeniser (x6) at a constant pressure of 15KPa. The cell debris was pelleted at 16,000 RPM and the supernatant used for further purification.

Column 1
Ni-NTA (5.0 ml volume in a gravity-flow column).
The clarified cell extract was incubated with 5.0 ml pre-equilibriated 50% Ni_NTA bead solution for 1 hour at 4°C with rotation after which it was passed through a glass column. The column was then washed with 30ml Binding Buffer (2 x 15ml) and 50 ml Wash Buffer (2 x15 ml). The protein was eluted with 25 ml of Elution Buffer in 5 x 5 ml fractions.

Column 2
Superdex s200 16/60 Gel Filtration.
A pool of the fractions (BB2+WB1+WB2+E1) was concentrated to a volume of 5 ml using a 10 kDa mwco concentrator and applied directly to 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 (20°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.

Extraction

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

A glycerol stock was used to inoculate 2X60 ml of TB media containing 50mg/ml kanamycin and 50 ìg/ml chloramphenicol, which was placed in a 37°C shaker overnight. The next day this starter culture was used to inoculate 12L of TB media (10 ml starter culture used per 1L) containing 50 ìg/ml kanamycin. When the OD600 reached approximately 1.0 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 16,000 RPM after which the supernatant was 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 15.23 mg/ml using a 10 kDa mwco concentrator.
Ligand
MassSpec: Expected mass: 46731.0 Da
Measured mass: 47649.9592 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 0.2M Magnesium Chloride -- 0.1M tris pH 8.5 -- 25%(w/v) PEG 3350.

NMR Spectroscopy:
Data Collection: Resolution: 1.77 Å
X-ray source: Diamond Light Source beamline IO4
Data Processing: