UPLC1

PDB:2B0O

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC060786
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal histag with thrombin cleavage site: mgsshhhhhhssglvprgs
Host:

Construct

Prelude:
Sequence:

GHDGEPHDLTKLLIAEVKSRPGNSQCCDCGAADPTWLSTNLGVLTCIQCSGVHRELGVRFSRMQSLTLDLLGPSELLLALNMGNTSF
NEVMEAQLPSHGGPKPSAESDMGTRRDYIMAKYVEHRFARRCTPEPQRLWTAICNRDLLSVLEAFANGQDFGQPLPGPDAQAPEELV
LHLAVKVANQASLPLVDFIIQNGGHLDAKAADGNTALHYAALYNQPDCLKLLLKGRALVGTVNEAGETALDIARKKHHKECEELLEQ
AQAGTFAFPLHVDYSWVISTE

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure: We prepared the seeds by inoculating freshly transformed E. coli cells (BL21 DE3) into 10 mL of LB medium. After overnight, 10 mLs were transferred to 900 ml of LB medium in the presence of 50 µg/ml of kanamycin at 37ºC and grown to an OD600 of 0.5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside at the final concentration of 1 mM and further grown for 3 hrs

Purification

Procedure
The supernatant was passed through DE52 (Whatman) column equilibrated with the binding buffer and then loaded onto 1 mL Ni-NTA column (Qiagen) equilibrated with the same binding buffer at 4 ºC. The Ni-NTA column was washed with 150 mL of the wash buffer (10mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol, 30 mM imidazole) and the protein was eluted with 15 mL of the elution buffer (10mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol, 250 mM imidazole). The eluate was dialyzed overnight against a buffer containing 10 mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol, 20

mM DTT and 10 uM ZnSulfate. The protein was concentrated using an Amicon Ultra centrifugal filter to the final concentration of 18.1 mg/mL.

Extraction

Procedure
Cultures were centrifuged and the cell pellets were suspended in 100 ml of the binding buffer (10 mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol, 5 mM imidazole, 10uM ZnSulfate) with a protease inhibitor cocktail (0.1 mM M benzamidine-HCl and 0.1 mM phenylmethyl sulfonyl fluoride) and flash frozen. The thawed cell pellet was lysed by a combination of 0.5% CHAPS (Sigma) and sonication. The lysate was centrifuged at 24,000 rpm for 30 min and the supernatant was used for subsequent steps of purification.
Concentration:
Ligand
MassSpec:
Crystallization: The supernatant was passed through DE52 (Whatman) column equilibrated with the binding buffer and then loaded onto 1 mL Ni-NTA column (Qiagen) equilibrated with the same binding buffer at 4 ºC. The Ni-NTA column was washed with 150 mL of the wash buffer (10mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol, 30 mM imidazole) and the protein was eluted with 15 mL of the elution buffer (10mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol, 250 mM imidazole). The eluate was dialyzed overnight against a buffer containing 10 mM Tris pH 7.5, 0.5 M NaCl, 5% glycerol, 20 mM DTT and 10 uM ZnSulfate. The protein was concentrated using an Amicon Ultra centrifugal filter to the final concentration of 18.1 mg/mL.
NMR Spectroscopy:
Data Collection:
Data Processing: