PECI

PDB:2F6Q

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:PECIA-s001
Entry Clone Source:MGC
SGC Clone Accession:
Tag:
Host:E.coli BL21 DE3

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmGFETLVVTSEDGITKIMFNRPKKKNAINTEMYHEIMRALKAASKDDSIITVLTGNGDYYSSGND
LTNFTDIPPGGVEEKAKNNAVLLREFVGCFIDFPKPLIAVVNGPAVGISVTLLGLFDAVYASDRATFHTPFSHLGQSPEGCSSYTFP
KIMSPAKATEMLIFGKKLTAGEACAQGLVTEVFPDSTFQKEVWTRLKAFAKLPPNALRISKEVIRKREREKLHAVNAEECNVLQGRW
LSDECTNAVVNFLSR

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:10 Âµl of a glycerol stock was inoculated into 5ml of LB medium (supplemented with Kanamycin, 50ug/ml) in a 15 ml culture tube and cultured at 37Â°C o/n in a shaking incubator (275 rpm). The starter culture was used to inoculate 100 ml of LB medium and was grown at 37Â°C (200 rpm). At an OD of 2.2 the culture was harvested and the cell pellet was washed twice with M9 minimal medium (Molecular Dimensions Ltd). The cells were resuspended and used to inoculate 1 liter of prewarmed minimal medium. Methionine synthesis was suppressed by addition of leucine, isoleucine and valine (dissolved as 50 mg/l for each aa) and lysine, threonine, and phenylalanine (100mg/l of each aa). Selenomethionine was added to a concentration of 25 mg/l, and the cultures were induced by supplementation with 1 mM IPTG. Cells were grown overnight at 18Â°C, collected by centrifugation and stored frozen until further use.

Purification

Procedure

Column 1 : Ni-affinity, HisTrap, 1 ml (GE Healthcare)
Buffers: Wash buffer: 500 mM NaCl, 5% Glycerol, 50 mM Tris-HCl pH 7.5, 30 mM Imidazole, 0.5 mM TCEP; Elution buffer: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 250mM Imidazole, 0.5 mM TCEP.

Column 2 : Gel filtration, Hiload 16/60 Superdex 200 prep grade 120 ml (GE Healthcare)
Buffers : GF buffer: 10 mM HEPES, pH 7.5, 500 mM NaCl, 5 % glycerol, 0.5 mM TCEP.

Procedure: The eluted fractions from the Ni-affinity Histrap columns were loaded on the gel filtration column in GF buffer at 0.7ml/min using an AKTA purifier system (GE Healthcare). Eluted proteins were collected in 1 ml fractions and concentrated using Vivaspin concentrators.

Extraction

Procedure
Lysis buffer: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 5 mM Imidazole , CompleteÂ® protease inhibitors (Roche, 1 tbl/50 ml). Frozen cell pellets were thawed and resuspended in a total volume of 30-40 ml of lysis buffer, and disrupted by sonication, and a supernatant containing the target protein was obtained by centrifugation.The clear supernantant was passed twice over a 2.5 ml Ni-NTA resin, washed and eluted with the specified buffers.
Concentration:
MassSpec:
Crystallization: Column 1 : Ni-affinity, HisTrap, 1 ml (GE Healthcare)
Buffers: Wash buffer: 500 mM NaCl, 5% Glycerol, 50 mM Tris-HCl pH 7.5, 30 mM Imidazole, 0.5 mM TCEP; Elution buffer: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 250mM Imidazole, 0.5 mM TCEP.

Column 2 : Gel filtration, Hiload 16/60 Superdex 200 prep grade 120 ml (GE Healthcare)
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NMR Spectroscopy:
Data Collection:
Data Processing: