CENTG1

PDB:2BMJ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:CENTG1A-s001
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with TEV cleavage site:
MHHHHHHSSGVDLGTENLYFQ*SH
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

SMRSIPELRLGVLGDARSGKSSLIHRFLTGSYQVLEKTESEQYKKEMLVDGQTHLVLI REEAGAPDAKFSGWADAVIFVFSLEDEN
SFQAVSRLHGQLSSLRGEGRGGLALALVGT QDRISASSPRVVGDARARALCADMKRCSYYETCATYGLNVDRVFQEVAQKVVTLRK
QQ QLLA

Vector:pLIC-SGC

Growth

Medium:
Antibiotics:
Procedure: From a frozen stock of BL-21(DE3) transformed with the CENTG1 construct a starter culture of 10 ml LB plus 100 µg/ml Amp was grown overnight at 37oC. This was used to inoculate 1 litre of LB the following morning. Cells were grown at 37oC until they reached an OD600 of 0.5. Protein expression was induced by the addition of 1 mM IPTG for a period of 12 hrs at 18oC. The cells were then spun down and resuspended in lysis buffer and frozen at -80oC. Lysis/binding buffer: 20 mM Tris pH 8.0, 200 mM NaCl, 5 % Glycerol, 10 mM imidazole.

Purification

Procedure
IMAC: Wash Buffer: 30 mM Tris pH8.0, 200 mM NaCl, 5 % Glycerol, 20 mM imidazole pH 8.0. Elute Buffer: 20mM Tris pH8.0, 200mM NaCl, 5 % Glycerol, 250 mM imidazole pH 8.0. Procedure: 5ml Ni-NTA agarose (50%) was placed in a BioRad drip column (2 columns for 4 L) and washed with 5 column volumes of Lysis/Binding Buffer (12.5ml). The resin was washed

once with 30ml column volumes of Lysis/Binding Buffer , followed by a wash Wash Buffer (12.5ml) again.Elute Buffer was applied to the resin (12.5ml) and 2.0 ml fractions collected.

S75 16/60: G.F. Buffer: 50 mM Tris pH 8.0, 150mM NaCl. Procedure : The column was pre-equilibrated with 2 column volumes of G.F. buffer at a flow rate of 1 ml/min. The elution fractions from the IMAC were concentrated to a final volume of 1.5 ml before loading onto the gel filtration column. The fractions that were identified on SDS-PAGE as purified CENTG1 were pooled and concentrated to 10 mg/mL using a Vivaspin 10 kD spin concentrator.

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TEV protease was added at 4 oC overnight to remove the hexahistidine tag. Any uncleaved protein was removed by passing the solution twice over Ni-NTA agarose beads. Finally the TEV protease cleaved CENTG1 was concentrated to a final concentration of 30 mg/mL.

Extraction

Procedure
After defrosting the cell pellet one EDTA-free protease table was dissolved before passing the cells through the Emulsiflex C5 high pressure homogeniser. The homogenised samples were transferred to a 50 centrifuge tube and PEI added to a final concentratio of 0.15 %. The homogenate was then centrifuged for 40 mins at 15,000rpm to remove all insoluble material.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals grew from a 1:1 mix of protein (50 mM Tris pH 8.0, 150 mM NaCl) and precipitant (0.1 M SPG pH 7.0, 30 % PEG1000, 0.5 % DMSO).
NMR Spectroscopy:
Data Collection:
Data Processing: