Entry clone source: genomic

Entry clone accession: gi 17545169

Vector: pET-30a. Details [PDF]; Sequence [FASTA] or [GenBank]

Tags and additions: C-terminal hexahistidine tag, no cleavage site.

Host: Bl-21(DE3)star

Entry clone accession/ sequence:
MKPPARKPRILNSDGSSNITRLGLEKRGW
LDDHYHDLLTVSWPVFITLITGLYLVTNA
LFALAYLACGDVIENARPGSFTDAFFFSV
QTMATIGYGKLIPIGPLANTLVTLEALCG
MLGLAVAASLIYARFTRPTAGVLFSSRMV
ISDFEGKPTLMMRLANLRIEQIIEADVHL
VLVRSEISQEGMVFRRFHDLTLTRSRSPI
FSLSWTVMHPIDHHSPIYGETDETLRNSH
SEFLVLFTGHHEAFAQNVHARHAYSCDEI
IWGGHFVDVFTTLPDGRRALDLGKFHEIA
Q

Growth medium, induction protocol: Cells were growth in LB plus 50 µg/ml kanamycin until an OD_{600}~ 1.1 before induction with 0.4 mM IPTG. The temperature was then decreased from 37oC to 25oC and the cells further cultured for 12 hrs.

Extraction buffer, extraction method: 50 mM Tris pH 8.0, 150 mM KCl, 250 mM sucrose, 10 mM $MgSO_4$. Before cell disruption with a high pressure homogeniser (Avestin C5) a Complete EDTA-free table and 5 µM pepstatin A were added. The lysate was centrifuged at 10,000g to remove cell debris. All membranes were dissolved with the addition of 30 mM decylmaltoside (DM) for 3 hrs at RT. Centrifugation at 45,000g removed any insoluble debris. The supernatant was then added to washed Talon Co2+ resin for batch binding. The sample was rotated gently for 1 hr at RT.

Column 1: Low pressure chromatography using Bio-Rad Econo column (2.5 cm x 13cm).

Buffers: (1) Wash I: 50 mM Tris pH 8.0, 100 mM KCL, 10 mM DM **. (2) Wash II:** 50 mM Tris pH 8.0, 500 mM KCL, 10 mM DM. **(3) Wash III :** 50 mM Tris pH 8.0, 150 mM KCL, 10 mM DM, 20 mM imidazole. **Elution buffer (EB):** 50 mM Tris pH 8.0, 150 mM KCL, 10 mM DM, 500 mM imidazole. **Procedure:** 10 column volumes of the wash buffers before elution with EB. 5 ml fractions were collected.

Column 2: Gel Filtration using Superdex 200 column

Buffers: 50 mM Tris pH 8.0, 150 mM KCl, 0.5 mM tridecylmaltoside. **Procedure:** The column was equilibrated with 50 ml of the running buffer.

Concentration: 10-15 mg/ml

Mass spec characterization: Expected 33,637 observed 33,606

Crystallisation: Home screen: 90 mM HEPES pH 7.5, 20 % PEG 400, 12.5 mM MgCl2, 14 mM Hega-10. For both intermediate states (IS1 and IS2) 50 mM spermine was added to the concentrated protein 8 hrs before crystallisation setup. For formation of IS2 10 mM CaCl2 was added after the crystals had formed.

Data Collection: Resolution: 2.8Å (1XL4), 2.6Å (1XL6); **X-ray source:** Synchrotron ESRF-BM14, single wavelength.