KirBac 3.1

PDB:1XL6

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi:17545169
Entry Clone Source:genomic DNA
SGC Clone Accession:
Tag:C-terminal hexahistidine tag, no cleavage site.
Host:BL21 (DE3) Star

Construct

Prelude:
Sequence:

MKPPARKPRILNSDGSSNITRLGLEKRGWLDDHYHDLLTVSWPVFITLITGLYLVTNALFALAYLACGDVIENARPGSFTDAFFFSV
QTMATIGYGKLIPIGPLANTLVTLEALCGMLGLAVAASLIYARFTRPTAGVLFSSRMVISDFEGKPTLMMRLANLRIEQIIEADVHL
VLVRSEISQEGMVFRRFHDLTLTRSRSPIFSLSWTVMHPIDHHSPIYGETDETLRNSHSEFLVLFTGHHEAFAQNVHARHAYSCDEI
IWGGHFVDVFTTLPDGRRALDLGKFHEIAQ

Vector:pET-30a

Growth

Medium:
Antibiotics:
Procedure: 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 37 ºC to 25 ºC and the cells further cultured for 12 hrs.

Purification

Procedure
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

Extraction

Procedure
50 mM Tris pH 8.0, 150 mM KCL, 250 mM sucrose, 10 mM MgSO4. 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.
Concentration:
Ligand
MassSpec: Expected 33,637 observed 33,606
Crystallization: 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.
NMR Spectroscopy:
Data Collection:
Data Processing: