BLVRA

PDB:2H63

Revision

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Entry Clone Accession:AU44-A5 Chlp; BC008456
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SGC Clone Accession:
Tag:N-terminal TEV-cleavable (at *) his-tag with the following sequence mhhhhhhssgvdlgtenlyfq*s
Host:BL21(DE3)-R3

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsMRKFGVV VVGVGRAGSVRMRDLRNPHPSSAFLNLIG FVSRRELGSIDGVQQISLEDALSSQEV
EV AYICSESSSHEDYIRQFLNAGKHVLVEYP MTLSLAAAQELWELAEQKGKVLHEEHVEL LMEEFAFLKKEVVGKDLLKGSLLF
TAGPL EEERFGFPAFSGISRLTWLVSLFGELSLV SATLEERKEDQYMKMTVCLETEKKSPLSW IEEKGPGLKRNRYLSFHFKSG
SLENVPNV GVNKNIFLKDQNIFVQKLLGQFSEKELAA EKKRILHCLGLAEEIQKYCCSRK*Q*RWI RIR

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Media: TB.
10ml of overnight culture was added into 1L TB with 50μg/ml of Kanamycin (totol 4L). The cells were cultured at 37°C until the OD reached 1.477 and then decreased the temperature to 18°C. IPTG was added at 0.5mM (final concentration) and kept the culture at 18°C for overnight.

Purification

Procedure
Column 1 : Ni-NTA
The column was packed by 4 ml of Ni-NTA slurry and equilibrated with 15 ml of binding buffer. The supernatant was loaded onto the column and the flow through was collected. The column was washed with 50 ml of binding buffer and then 50 ml of washing buffer. The protein was eluted with 12 ml of elution buffer and collected by 1.5 ml fractions.

Column 2 : Superdex 200 Hiload 16 60
AKTA Purifier was used. After runing the SDS gel, 8 fractions were combined together for TEV cleavage.

Column 3: Ni-NTA
His-tag was cleaved by TEV protease. The sample was loaded onto the column (packed from 0.4 ml of Ni-NTA slurry). The flow through was collected and the column was then washed with 3 ml of the buffer (also collected).

Final concentration : 29.73 mg/ml

Enzymatic treatment : 200µl of TEV protease were added into the sample after gel filtration .The sample was incubated at 4°C overnight

Extraction

Procedure
The cells were harvested by centrifugation at 4,000 g for 10 min. The pellet from 1 L culture was resuspended in 25 ml of extraction buffer. The sample was homogenized by using the EmulsiFlex-05 homogenizer (Glen Creston) and then centrifuged at 37505 g. The supernatant was kept for further purification
Concentration:
Ligand
MassSpec:32976
Crystallization: Crystals were grown by vapour diffusion at 4°C in 150 nl sitting drops. NADPH (or NADP +) to a final concentration of 5 mM was added to the protein just prior to crystallisation. The drops were prepared by mixing 50 nl of protein solution and 100 nl of buffer consisting of 0.2 M MgCl 2 (or 0.2M NaCl) , 0.1 M Bis-Tris pH 5.5 and 25% PEG 3350.
NMR Spectroscopy:
Data Collection: Resolution: 2.7Å; X-ray source: Synchrotron SLS -X10
Data Processing: