DIRAS2

PDB:2ERX

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:DIRAS2A-s001
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal, TEV cleavable hexahistidine tag
Host:BL21(DE3)R3 phage resistant strain

Construct

Prelude:
Sequence:

SMSNDYRVAVFGAGGVGKSSLVLRFVKGTFRESYIPTVEDTYRQVISCDKSICTLQITDTTGSHQFPAMQRLSISKGHAFILVYSIT
SRQSLEELKPIYEQICEIKGDVESIPIMLVGNKCDESPSREVQSSEAEALARTWKCAFMETSAKLNHNVKELFQELLNLEKRRTVSL
Q

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure: Freshly transformed E. coli cells was used to inoculate 1 litre of TB plus 50 µg/mL kanamycin. When OD600 reached ~1.0 the temperature was shifted down from 37°C to 25°C for 1 hour before induction with the addition of 1 mM IPTG. Protein expression was allowed to carry on for a further 4 hours before harvest.

Purification

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

Buffers: Wash buffer I (WB1): 50 mM Tris pH 8.0, 150 mM NaCl, 5 % Glycerol, 10mM MgCl2, 10mM Imidazole pH 8.0. Wash Buffer II (WBII): 50 mM Tris pH 8.0, 150 mM NaCl, 5 % Glycerol, 10 mM MgCl2, 30 mM Imidazole pH 8.0. Elution buffer (EB): 50 mM Tris pH 8.0, 150 mM NaCl, 5 % Glycerol, 10 mM MgCl2, 250 mM Imidazole pH 8.0.

Procedure: Total volume of Ni-NTA added to BioRad drip column: 4 mLs (50%). Resin washed with 12.5 mL of WB1. The supernatent was applied to a column using 5 mL pipette and allowed

to pass over the resin. The flow through was collected in a 50 mL falcon tube and applied once more to the column. Two wash steps followed. Wash with 12.5 mL of WBI. Wash with 12.5 mL column vols of WBII. Elute with 14 mLs of EB into 7x2 mL fractions. At this stage the purity of the protein was greater than 95 % based on SDS - PAGE analysis. The C-terminal hexahistidine tag was removed by TEV protease treatment. The TEV protease, a hexahistidine-tagged construct, was over-expressed and purified in-house to a final concentration of 2.5 mg/mL.

Extraction

Procedure
Cell Lysis - Resuspension buffer (RS): 50 mM Tris pH 8.0, 150 mM NaCl, 5 % Glycerol, 10 mM Imidazole pH 8.0, 10mM MgCl2 . 1 proteinase inhibitor tablet (EDTA free) was added to 10mL Resuspension Buffer. This was used to syspend the pellet before homogenisation. Total vol:45 mLs (estimate) Cell breakage: 5 passes through the Emulsiflex C5 high pressure homogeniser. Total vol: 50 mLs (estimate). Centrifuge for 40 mins at 16000 rpm and 4°C to remove cell debris. Discard pellet.
Concentration:
Ligand
MassSpec:
Crystallization: Column 1 : Low pressure chromatography using Bio-Rad Econo column (2.5 cm x 13 cm).

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NMR Spectroscopy:
Data Collection:
Data Processing: