PAK6

PDB:2C30

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|9910476
Entry Clone Source:MGC
SGC Clone Accession:
Tag: Tag sequence: mhhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*) N-terminal his6 tag.
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

```
MQDPTVAKGALAGEDTGVVTHEQFKAALRMVVDQGDPRLLLDSYVKIGEGSTGIVCLAREKHSGRQVAVKMMDLRKQQRRELLFNEV
VIMRDYQHFNVVEMYKSYLVGEELWVLMEFLQGGALTDIVSQVRLNEEQIATVCEAVLQALAYLHAQGVIHRDIKSDSILLTLDGRV
KLSDFGFCAQISKDVPKRKSLVGTPYWMAPEVISRSLYATEVDIWSLGIMVIEMVDGEPPYFSDSPVQAMKRLRDSPPPKLKNSHKV
SPVLRDFLERMLVRDPQERATAQELLDHPFLLQTGLPECLVPLIQLYRKQTST
```

Vector:pLIC-SGC1

Growth

Medium:
Antibiotics:
Procedure:1ml from a 10 ml overnight culture containing 50 µg/ml kanamycin was used to inoculate 1 litre of LB containing 50 µg/ml kanamycin. Cultures were grown at 37°C until the OD600 reached ~0.3 then the temperature was adjusted to 18°C. Expression was induced for 4 hours using 1 mM IPTG at an OD600 of 0.8. The cells were collected by centrifugation and the pellet resuspended in binding buffer and frozen. Binding buffer: 50mM HEPES pH 7.5; 500 mM NaCl; 5 mM imidazole, 5% glycerol.

Purification

Procedure
Column 1: Ni-affinity. Ni-NTA (Qiagen), 5 ml of 50% slurry in 1.5 x 10 cm column, washed with binding buffer.

Extraction

Procedure
Frozen pellets were thawed and cells lysed using a high pressure cell disrupter. The lysate was centrifuged at 50,000 rpm for 40 minutes and the supernatant collected for purification.
Concentration:
Ligand
MassSpec:
Crystallization: Protein concentration, 10 mg/ml. Well solution: 1.60M magnesium sulfate, 0.1M MES 6.5.
NMR Spectroscopy:
Data Collection:
Data Processing: