Entry Clone Source: MGC

Entry Clone Accession: IMAGE:5553184

SGC Construct ID: ARHGAP15A-c002

GenBank GI number: gi|28466979

Vector: pNIC28-Bsa4. Details [PDF]; Sequence [FASTA] or [GenBank]

Tags and additions: Tag sequence: `mhhhhhhssgvdlgtenlyfq*s(m)` TEV-cleavable (*) N-terminal his6 tag.

Final protein sequence (tag sequence in lowercase):

```
mhhhhhhssgvdlgtenlyfq*sMRPSLK
TLQEKGLIKDQIFGSHLHKVCERENSTVP
WFVKQCIEAVEKRGLDVDGIYRVSGNLAT
IQKLRFIVNQEEKLNLDDSQWEDIHVVTG
ALKMFFRELPEPLFPYSFFEQFVEAIKKQ
DNNTRIEAVKSLVQKLPPPNRDTMKVLFG
HLTKIVAKASKNLMSTQSLGIVFGPTLLR
AENETGNMAIHMVYQNQIAELMLSEYSKI
FGSE
```

Host:BL21(DE3)-R3-pRARE2 (previously known as Rosetta)

Growth medium, induction protocol: 10ml from a 50 ml overnight culture containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol was used to inoculate 1 liter of TB media containing the same concentration of antibiotics. Cultures were grown at 37°C until the OD_{600} reached ~1.5. After that the temperature was adjusted to 22°C. Expression was induced over night using 0.5 mM IPTG. 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% glycerol, 5 mM imidazole.

Extraction method: Cell pellets were lysed by C5 high pressure homogenizer (Avestin). The lysate was centrifuged at 17500 rpm for 60 minutes and the supernatant collected for purification.

Column 1: Ni-affinity chromatography.

Buffers: Binding buffer: Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 5mM Imidazole. **Wash buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM Imidazole, 5% glycerol. **Elution buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 250 mM imidazole, 5% Glycerol.

Procedure: 5 ml of 50% Ni-NTA slurry (Qiagen) was applied to a 1.5 x 10 cm gravity column. The column was equilibrated with 50 ml binding buffer. **The lysate was applied to the** column which was subsequently washed with 50 ml wash buffer 1 and 2. The protein was eluted by gravity flow by applying 15 ml portions of elution buffer containing 250 mM imidazole. The eluted protein was analyzed by SDS-PAGE. DTT was added to the protein sample to a final concentration of 5mM.

Column 2: Size exclusion chromatography (Superdex S200, 60 x 1cm)

SEC-Buffers: 50 mM Hepes, pH 7.4, 500 mM NaCl, 0.5 mM TCEP.

Procedure: The fractions eluted of the Ni-affinity chromatography were concentrated to about 2 mls using Centricon concentrators (10kDa cut off). The concentrated protein was applied to a Superdex S200 column equilibrated in SEC buffer at a flow rate of 0.8 ml/min. Eluted fractions were >95% pure as judged by SDS-PAGE.

Protein concentration: Centricon with a 10 kDa cut off in SEC-buffer.

Mass Spectrometry Characterisation: The mass of the protein was calculated to be 24472.1 Da and experimentally determined mass was 24472.5 Da for the His tag cleaved protein. The identity of the

protein was reconfirmed to be correct by DNA sequencing both DNA strands of this expression construct.

Crystallization: The protein was concentrated in gel filtration buffer to a protein concentration of 17.48 mg/ml. Crystallizations were carried out using sitting drop vapor diffusion at 20°C. Crystals appeared in 150nl drops composed of equal volume of protein (17.5mg/ml) and reservoir solution containing 2.4 M sodium malonate 7.0.

Data Collection: Crystals were cryo-protected using the well solution supplemented with 20% Ethylene Glycol and flash frozen in liquid nitrogen.**Resolution:** 2.2 Å; Diffraction data were collected at the SLS beam line SAX10.