Entry Clone Source: MGC

Entry Clone Accession: IMAGE:5296057

SGC Construct ID: GIPC2A-c011

GenBank GI number: gi|41393579

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

Tags and additions: N-terminal TEV-cleavable (at *) his-tag with the following sequence
mhhhhhhssgvdlgtenlyfq*s

Protein sequence (sequence tag in lowercase):
mhhhhhhssgvdlgtenlyfqsMKGIEKE
VNVYKSEDSLGLTITDNGVGYAFIKRIKD
GGVIDSVKTICVGDHIESINGENIVGWRH
YDVAKKLKELKKEELFTMKLIEPKKSSEA

Host: BL21(DE3)-R3-pRARE2

Growth medium, induction protocol: An overnight culture (10 ml) was used to inoculate 1L TB medium (supplemented with 50 µg/ml of Kanamycin and 34 µg/ml of chloramphenicol). The cells were cultured at 37°C with vigorous shaking (160 rpm) until the culture reached an OD_{600} of 1.5. At that point temperature was reduced to 18°C, and cells were induced with IPTG at a concentration of 0.2 mM, and cultured o/n. The following day the cells were harvested at 9000 x g for 10 minutes and the cell pellet of 1L was resuspended in 25 ml of lysis buffer and stored at -80°C until further use. Lysis buffer : 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 20 mM imidazole, Complete EDTA-free protease inhibitor (Roche, 1tablet / 50ml).

Extraction method: The resuspended pellet was thawed and lysed in a high pressure homogeniser and then centrifuged at 4°C for 45 minutes at 48 000 x g.

Column 1: Ni-affinity, HisTrap, 1 ml (GE/Amersham Biosciences)

Buffers: Binding buffer: 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 20 mM imidazole, 0.5mM TECP; **Wash Buffer:** 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 40 mM imidazole,0.5mM TECP; **Elution Buffer:** 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 250 mM imidazole,0.5mM TECP.

Procedure: AKTA Xpress Affinity/Gel Filtration. The centrifuged cell extract was loaded on the column at 1 ml/minute on an AKTA-express system (GE/Amersham). The column was then washed with 10 column volumes of lysis buffer, 10 column volumes of wash buffer, and then eluted with 1 column volume elution buffer at 1 ml/min. The eluted peak of A280 nm was automatically collected.

Column 2: Size Exclusion Chromatography (SEC) Hiload 16/60 Superdex 200 prep grade 120 ml (GE/Amersham Biosciences).

Buffers: Gelfiltration bufffer: 10 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 0.5mM TCEP.

Procedure: AKTA Xpress Affinity/Gel Filtration. The eluted fractions from the Ni-affinity Histrap columns were loaded on the gel filtration column in GF buffer at 1ml/min. Eluted protein was collected in 1.8 ml fractions in a 96 well bloc and analyzed by SDS-PAGE. Positive fractions were pooled for TEV cleavage.

TEV cleavage: The gel filtration fractions containing GIPC2A were pooled (in total 14.25 mg) and 130 µl of TEV protease solution 6 mg/ml) was added. The digestion was left overnight at 4°C and cleavage was examined by SDS page, before passing the reaction mixture through Ni-NTA resin.

Column 3: Ni-NTA

Procedure: The combined GIPC2A (Tev cleaved) samples (identified by SDS PAGE) were loaded on 1 ml Ni-NTA resin for further purification.
Concentration: The eluted flow through fractions from the Ni-NTA resin were concentrated using centricons with 5 kDa cut off (Amicon Ultra 5k, Millipore) to 5.1 mg/ml.
Mass spectrometry characterization: LC-ESI-MS TOF confirmed the correct mass of 10614 Da expected for this construct of GIPC2A.
Crystallization: Crystals were grown by vapour diffusion in sitting drops at 4°C. A sitting drop consisting of 100 nl protein and 50 nl well solution was equilibrated against well solution containing 2M ammonium sulphate and 2.5% (v/v) isopropanol. Crystals were cryo protected in 30% glycerol and flash-cooled in liquid nitrogen.
Data Collection: Resolution: 2.60 Å; **X-ray source:** Synchrotron SLS-X10SA, single wavelength.