Entry clone source: MGC

Entry clone accession: IMAGE:4706281

SGC Construct ID: ACADSBA-c003

GenBank GI number: gi|4501859

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:
mhhhhhhssgvdlgtenlyfq*smAPLQT
FTDEEMMIKSSVKKFAQEQIAPLVSTMDE
NSKMEKSVIQGLFQQGLMGIEVDPEYGGT
GASFLSTVLVIEELAKVDASVAVFCEIQN
TLINTLIRKHGTEEQKATYLPQLTTEKVG
SFCLSEAGAGSDSFALKTRADKEGDYYVL
NGSKMWISSAEHAGLFLVMANVDPTIGYK
GITSFLVDRDTPGLHIGKPENKLGLRASS
TCPLTFENVKVPEANILGQIGHGYKYAIG
SLNEGRIGIAAQMLGLAQGCFDYTIPYIK
ERIQFGKRLFDFQGLQHQVAHVATQLEAA
RLLTYNAARLLEAGKPFIKEASMAKYYAS
EIAGQTTSKCIEWMGGVGYTKDYPVEKYF
RDAKIGTIYEGASNIQLNTIAKHIDAEY

Host : BL21(DE3)-R3/pRARE

Growth medium, induction protocol: An overnight culture (10 ml) was used to innoculate 1L TB medium (supplemented with 50 µg/ml of Kanamycin). The cells were cultured in 6 litres 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 0.5 mM, and cultivated further for 16 hours. Cells were harvested at 6000 rpm for 10 minutes and the cell pellet was resuspended in 25 ml of lysis buffer and stored at -20°C until further use. Lysis buffer: 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 5 mM imidazole, Complet EDTA-free protease inhibitor (Roche, 1tbl/50ml).

Extraction method : The resuspended pellet was thawed and homogenised by using Emulsiflex-C5 homogenizer (Avestin) and then centrifuged at 4°C in Beckman JA-17 rotor at 16000 rpm for 45 min.

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

Buffers: Binding buffer : 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 5 mM imidazole,0.5mM TECP; **Washing Buffer:** 500 mM NaCl, 5% glycerol, 50 mM HEPES pH 7.5, 30 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 cell extract was loaded on the column at 0.8 ml/minute on an AKTA-express system (GE/Amersham). The column was then washed with 10 volumes of lysis buffer, 10 volumes of wash buffer, and then eluted with elution buffer at 0.8 ml/min. The eluted peak of A280nm was automatically collected.

Column 2 : Hiload 16/60 Superdex 200 prep grade 120 ml (GE/Amersham Biosciences)

Buffers : Gel filtration bufffer: 10 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 0.5mM TECP.

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 0.80 ml/min. Eluted protein was collected in 2 ml fractions in a 96well palate.

Concentration : The protein was concentrated to 30.1 mg/ml by using Milipore, Amicon Ultra 30k concentrator.
Mass spec characterization : The experimentally determined mass of ACADSBA was 44423.7 Da, which corresponds to the theoretical mass.
Crystallization: Crystals were grown by vapor diffusion in sitting drops at 20°C. Before setting up the experiment, FAD and acetoacetyl-CoA were added to the protein to final concentrations of 50 µM and 10 mM respectively. A sitting drop consisting of 100 nl protein and 50 nl well solution was equilibrated against well solution containing 30% PEG 6000, 0.15 M ammonium chloride pH 6.3, 10% ethylene glycol. The crystal was transferred to a cryo-protectant comprised of well solution supplemented with 15% ethylene glycol before flash-cooling in liquid nitrogen.
Data acquisition and analysis: Resolution: 2.0 Å; **X-ray source:** Synchrotron SLS -X10SA.