SORBS2

PDB:4IGZ

Revision

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Entry Clone Accession:NP_066547
Entry Clone Source:
SGC Clone Accession:SORBS2, YTC002E04
Tag:N-terminal tag: MKIEEHHHHHHSSGRENLYFQG
Host:BL21 (DE3)_V2R

Construct

Prelude:
Sequence:

mkieehhhhhhssgrenlyfqgGAAQPAMAQGALLPAKAVYDFKAQTSKELSFKKGDTVYILRKIDQNWYEGEHHGRVGIFPISYVE
KLTGSAAALRTGEAYLRYVDAAA

Vector:pET28a-MHL

Growth

Medium:TB
Antibiotics:
Procedure:A 250 mL flask containing LB (Sigma L7658) supplemented with 50 ug/ mL kanamycin (BioShop Canada KAN 201) was inoculated from a glycerol stock of the bacteria. The flask was shaken overnight (16 hours) at 250 rpm at 37 °C.

Using the Lex system, a 2L bottle (VWR 89000-242) containing 1800 mL of TB (Sigma T0918) supplemented with 1.5% glycerol, 50 ug/ mL kanamycin and 800 ul antifoam 204 (Sigma A-8311) was inoculated with 30 mL overnight LB culture, and incubated at 37 °C. The temperature of the media was reduced to 15 °C one hour prior to induction and induced at OD (600) = 2 with 250 uM isopropyl-thio-β-D-galactopyranoside (BioShop Canada IPT 001). Cultures were aerated overnight (16 hours) at 15 °C, and cell pellets collected by centrifugation and frozen at -80 °C.

Purification

Procedure
IMAC: Unclarified lysate was centrifuged at 16000 rpm for 1 hours at 4 degree. The supernatant was mixed with 4 mL of Ni-NTA superflow Resin (Qiagen) per 200 mL lysate. The mixture was incubated with mixing for at least 30 minutes at 4 ºC. The mixture was than loaded onto an empty

comLum (BioRad) and washed with 40 mL wash buffer. Samples were eluted from the resin by exposure to 2-3 column volumes (approx. 10-15 mL) of elution buffer. Concentration of eluted protein was estimated by OD280. TEV protease was added at 1 mg per 20 mg of eluted protein and dialyzes against gel filtration buffer overnight to remove His-tag.

Gel filtration chromatography: An 16x60 column (GE Healthcare) packed with HighLoad Superdex 75 resin (GE Healthcare) was pre-equilibrated with gel filtration buffer for 1.5 column volumes using an AKTA explorer (GE Healthcare) at a flow rate of 1 mL/min. The dialyzed sample from the IMAC step (approx. 3 mL, after concentrating by 15 mL concentrators (Amicon Ultra-15, UFC900524, Millipore)) was loaded onto the column at 1 mL/min, and 2mL fractions were collected into 96-well plates (VWR 40002-012) using peak fractionation protocols. Fractions observed by a UV absorption chromatogram to contain the protein were pooled.

Extraction

Procedure
Frozen cell pellet contained in bags (Beckman 369256) obtained from 2L of culture were thawed by soaking in warm water. Each cell pellet was resuspended in 200 mL lysis buffer and homogenized using an Ultra-Turrax T8 homogenizer (IKA Works) at maximal setting for 30-60 seconds per pellet. Cell lysis was accomplished by sonication (Virtis408912, Virsonic) on ice: the sonication protocol was 10 sec pulse at frequency8.5, 10 second rest, for 10 minutes total sonication time per pellet.
Concentration: Purified proteins were concentrated using 15 mL concentrators with a 5,000 molecular weight cut-off (Amicon Ultra-15, UFC900524, Millipore) at 3750 rpm, typically resulting in a final concentration around 20 mg/mL.
Ligand
MassSpec:
Crystallization: Diffraction quality crystals were grown using the following protocol: 28% PEG-2000, 0.1 M Bis-Tris, pH 6.5, vapor diffusion, sitting drop, temperature 298K.
NMR Spectroscopy:
Data Collection:
Data Processing: