SIR2A

PDB:3JWP

Revision

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Entry Clone Accession:XP_001350011
Entry Clone Source: Cloned from Pf3D7 gDNA
SGC Clone Accession:PF13_0152:K10-M273:MAC02W-E01
Tag:
Host:BL21-(DE3)-V2R-pRARE2

Construct

Prelude:
Sequence:

KKDTQSITLEELAKIIKKCKHVVALTGSGTSAESNIPSFRGSSNSIWSKYDPRIYGTIWGFWKYPEKIWEVIRDISSDYEIEINNGH
VALSTLESLGYLKSVVTQNVDGLHEASGNTKVISLHGNVFEAVCCTCNKIVKLNKIMLQKTSHFMHQLPPECPCGGIFKPNIILFGE
VVSSDLLKEAEEEIAKCDLLLVIGTSSTVSTATNLCHFACKKKKKIVEINISKTYITNKMSDYHVCAKFSELTKVANILKGSSEKNK
KIM

Vector:pET15-MHL-LIC

Growth

Medium:M9
Antibiotics:
Procedure: Selenomethionyl proteins were produced in BL21-(D3) - a strain not auxotrophic for methionine- using feedback inhibition of methionine biosynthesis. On day 1, one colony of the transformed E. coli BL21-(DE3)-V2R-pRARE2 was transferred to 5 mL LB medium supplemented with carbenicillin (100 µg/mL) and chloroamphenicol (34 µg/mL) in a 10 ml tube and grown at 37 °C, 220 rpm overnight. On day 2, 100 ml of freshly prepared "A" solution, supplemented with "B", "C", glycerol and antibiotics were inoculated with 0.5 ml of the LB culture and grown overnight in a 200 ml flask at 37°C, 220 rpm. On day 3, 1.8 L/ 2 L bottle of freshly prepared "A" solution, supplemented with "B", "C", glycerol, antibiotics and 0.5 ml antifoam A204 (Sigma) were inoculated with 20 ml of the flask cultures and grown in the LEX system at 37°C for ~ 4 h until OD600 ≈ 1.2. Then "D" was added, immediately the cultures were cooled down to 20°C (the cooler was set to 18°C). After 15 min IPTG was added and induced overnight. In the morning of day 4, the OD was taken and the cells harvested.

The solutions were prepared according to the instructions of the M9 SeMET High-Yield growth media kit package (MD045004-50L, Orion enterprise inc, Northbrook, IL, USA). Amendments (final amounts) per liter, added in a sterile manner to 900 - 950 ml autoclaved water:

"A": MD045004A (Na/K/P/C/N sources + non-inhibitory amino acid cocktail: EDRHAPGSQNW), one pouch for 1 Liter cell culture, added just before growth: 6 g Na2HPO4, 3 g KH2PO4, 1 g NH4Cl, 0.5 g NaCl, 4.4 g glucose, non-inhibitory amino acids 200 mg each (1-glutamate, 1-aspartate, 1-arginine, 1-histidine, 1-alanine, 1-proline, 1-glycine, 1-serine, 1-glutamine, 1-asparagine, 1-tryptophane)

"B": MD045004B (Mineral supplements), stored at 4°C, light avoided. 10 ml for 1 Liter cell culture: 5 mg EDTA, 430 mg MgCl*6 H2O, 5 mg MnSO4, 10 mg NaCl, 1 mg FeSO4*7H2O, 1 mg Co(NO3)2*6H2O, 11 mg CaCl2, 1 mg ZnSO4*7H20, 0.1 mg CuSO4*5H20, 0.1mg AlK(SO4)2, 0.1mg H3BO3, 0.1 mg Na2MoO4*2H20, 0.01 mg Na2SeO3, 0.1 mg Na2WO4*2H20, 0.2 mg NiCl2*6H2O.

"C": MD045004C (Vitamins), added to 50 ml of water and mixed, stored at 4°C for short term, at -20°C for long time use, light avoided after dissolution. 1 ml for 1 Liter cell culture: 1 ug thiamine (vitamin B1), 2.7 ug vitamin B12.

1 ml each of 1000 x stock solutions of antibiotics: 100 mg/ml carbenicillin, 34 mg/ml chloroamphenicol.

10 ml of 50% autoclaved glycerol

"D": MD045004D (inhibitory amino acid cocktail: VILKTF + SeMet). One pouch dissolved in 250 ml. 20 ml for 1 Liter cell culture: 25 mg each of 1-valine, 1-isoleucine, 1-leucine, 1-lysine, 1-threonine, 1-phenylalanine and 15 mg of selenomethionine.

"E": For MD045004E (IPTG). One pouch dissolved in 50 ml of water. 1 ml for 1 Liter cell culture. Final concentration: 1 mM isopropylthio-β-d-galactoside (IPTG).

Purification

Procedure

The cleared lysate of 6 L growth was loaded onto a 2.5 mL Ni-NTA (Qiagen) column (pre-equilibrated with Binding Buffer) at approximately 1.5-2.0 mL/min. The Ni-NTA column was then washed with 200 mL Wash Buffer A and 100 ml Wash Buffer B at 2-2.5 mL/min. After washing, the protein was eluted with Elution Buffer at approximately 2 drops per minute. The eluted sample was applied to a Sephadex S200 26/60 gel filtration column pre-equilibrated with Gelfiltration Buffer. The fractions corresponding to the eluted protein peak were collected. The his-tag was cleaved by TEV protease 12 mg/ml, activity 1:50 in the presence of 5 mM TCEP. After confirmation by Mass Spec, the cut protein was separated from the His-Tag and TEV protease on a 0.5 ml Ni-NTA (Qiagen) column (pre-equilibrated with Cut Buffer A). After eluting a first batch of cut protein with lower purity with Cut buffer A, a second batch of purer E1 protein was eluted with Cut Buffer B at approximately 2 drops/min which was later used to set up crystal trials. The buffer was exchanged with gelfitration buffer using a 15 mL Amicon Ultra centrifugal filter device (Millipore), then the sample was concentrated. The protein sample identity and purity were evalulated by mass spectroscopy and SDS-PAGE gel after Ni-NTA affinity, size exclusion chromatography and cutting. The concentrated protein was stored at 4 degC. For long term storage, the protein was flash frozen and stored at -80 degC.

Extraction

Procedure

The culture was harvested by centrifugation. Pellets from 4 L of culture were resuspended to approximately 30 mL/L of cell culture in Binding Buffer with the addition of protease inhibitors (1 mM benzamidine and 1 mM phenylmethyl sulfonyl fluoride (PMSF)). Resuspended pellets stored at -80 oC were thawed overnight at 4 °C on the day before purification. Prior to sonication, each pellet from 1 L of culture was pretreated with 0.5 % CHAPS and 500 units of benzonase for 40 minutes at room temperature. After 6 minutes sonication, the cell lysate was centrifuged using a Beckman JLA-16.250 rotor at 16000 rpm for 1 h at 4 degC.

Concentration:

Ligand

MassSpec:

Crystallization:hanging drop vapor diffusion. 20% sucrose plus 80% (17% PEG3350, 0.1M Na Citrate Tribasic dehydrate, pH5.8 and 0.1% b-OG) at temperature 293K.

NMR Spectroscopy:

Data Collection:

Data Processing: