RABGDI

PDB:3P1W

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source:
SGC Clone Accession:
Tag:
Host: Ros-Ox

Construct

Prelude:
Sequence:

Vector:p15-mhl

Growth

Medium:TB
Antibiotics:
Procedure:PF13_0119 was expressed in *E. coli* BL21-(DE3)-Rosetta-Oxford cells in Terrific Broth (TB) in the presence of ampicillin/chloramphenicol (50 µg/mL and 25 µg/mL respectively). A single colony was inoculated into 10 mL of LB with of ampicillin/chloramphenicol (50 µg/mL and 25 µg/mL respectively) in a 50 mL Falcon tube and incubated with shaking at 250 rpm overnight at 37 °C. The culture was transferred into 50 mL of TB with 50 µg/mL ampicillin in a 250 mL shaking flask and incubated at 37 °C for 3 hours. Then the culture was transfered into 1.8 L of TB with 50 µg/mL kanamycin and 0.3 mL of antifoam (Sigma) in a 2 L bottle and cultured using the LEX system to an OD_{600} of ~5, cooled to 15 °C, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 °C.

Purification

Procedure
The cleared lysate was loaded onto a column prepacked with 10 g DE52 (Whatman) anion exchange resin (previously activated with 2.5 M NaCl and equilibrated with Binding Buffer); and subsequently onto a 2.5 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1 mL/min. The volume of the Ni-NTA resin was pre-determined by the predicted protein yield from test expression analysis. After the lysate was loaded, the DE52 was further washed with 20 mL of Binding Buffer. The Ni-NTA column was then washed with 200 mL of

Wash Buffer at 3 mL/min. After washing, the protein was eluted with 15 mL of Elution Buffer. The eluted protein was dialyzed overnight at 4°C into 4 L of Crystal Buffer (10 mM HEPES, pH 7.5, 500 mM NaCl). Reducing agent (TCEP) was added to the sample at a final concentration of 2mM. The sample was concentrated to a final volume of 1ml and the protein identity was evaluated by SDS-Page and mass spectroscopy. The concentrated protein was stored at 4°C.

Extraction

Procedure
The culture was harvested by centrifugation. Pellets from 2 L of culture were resuspended to approximately 40 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 °C were thawed overnight at 4 °C on the day before purification. Prior to mechanical lysis, each pellet from 1 L of culture was pretreated with 0.5% CHAPS, 0.5mg/ml Egg white lysozyme and 500 units of benzonase for 40 minutes at room temperature. Cells were mechanically lysed with a microfluidizer (Microfluidizer Processor, M-110EH) at approximately 18000 psi; and the cell lysate was centrifuged using a Beckman JA-25.50 rotor at ~75000 x g (24000 rpms) for 20 minutes at 10 °C.
Concentration:
Ligand
MassSpec:
Crystallization: The protein was crystallized at 18 °C in 25% PEG 3350 0.2 M NaCl, 100 mM HEPES, pH 7.5 using the hanging drop vapor diffusion method.
NMR Spectroscopy:
Data Collection:
Data Processing: