FXR2

PDB:3H8Z

Revision

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Entry Clone Accession: BC067272
Entry Clone Source:MGC AT94-B9,
SGC Clone Accession:
Tag:N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgrenlyfq*g.
Host:*E. coli* BL21(DE3)-V2R-pRARE2

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfqgLPVEVRGSNGAFYKGFVKDVHEDSVTIFFENNWQSERQIPFGDVRLPPPADYNKEITEGDEVEVYSRAN
EQEPCGWWLARVRMMKGDFYVIEYAACDATYNEIVTLERLRPVNPNPLATKGSF

Vector:pET28-MHL

Growth

Medium:M9 SeMET groth media (Medicilon Inc).
Antibiotics:
Procedure:A fresh transformation was used to inoculate 20 mL LB media containing 50 µg/mL kanamycin and 30 µg/mL chloramphenicol . The culture was grown overnight at 37°C with shaking. The next day this starter culture was used to innoculate 2L of M9 SeMET growth medium. The culture was grown in LEX at 37°C to OD600 of 2.3. Methionine biosynthesis inhibition and IPTG-based induction were carried out according to the manufacturer's protocol. The temperature was reduced to 14°C and the culture was incubated for a further 18 hours before harvesting the cells.

Purification

Procedure
Column 1: Affinity purification, open Ni-NTA column Procedure: The supernatant was incubated with 6mL of 50% slurry Ni-NTA beads by rocking. After 1 hour incubation at 4ºC, the beads were washed with 50 mL of lysis buffer. The protein was eluted using ~20mL EB. Column 2: Size Exclusion, HiLoad 16/60 Superdex 75 Prep Grade Procedure: The eluent from from the

NiNTA column was concentrated and loaded onto the size exclusion column in at 1 mL/min, fraction size 7mL. The fractions containing protein were identified on a SDS-PAGE gel.

Extraction

Procedure
Cells were harvested by centrifugation and pellets were stored in -80ºC. Prior to purification, the cell pellet was resuspended in lysis buffer. Cells were disrupted by sonication (10 minutes) and samples were centrifuged for 60 min at 70000 g.
Concentration:10 mg/ml.
Ligand
MassSpec:
Crystallization:25% PEG 3350, 0.1 M Tris pH 7.0, 0.2M MgCl2, 10 mM DTT by hanging-drop vapour diffusion. The drop was prepared by mixing 2.5 microL protein with 1 microL of reservoir solution. Crystals appear after a minimum period of 2 weeks.
NMR Spectroscopy:
Data Collection:
Data Processing: