EPHA3

PDB:2GSF

Revision

Revision Type:created
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Entry Clone Accession:
Entry Clone Source:MGC
SGC Clone Accession:epha3.577.947:F8; plate SDC033 F8
Tag:
Host:BL21 (DE3)

Construct

Prelude:
Sequence:

gsDEKRLHFGNGHLKLPGLRTYVDPHTYEDPTQTVHEFAKELDATNISIDKVVGAGEFGEVCSGRLKLPSKKEISVAIKTLKVGYTE
KQRRDFLGEASIMGQFDHPNIIRLEGVVTKSKPVMIVTEYMENGSLDSFLRKHDAQFTVIQLVGMLRGIASGMKYLSDMGYVHRDLA
ARNILINSNLVCKVSDFGLSRVLEDDPEAAYTTRGGKIPIRWTSPEAIAYRKFTSASDVWSYGIVLWEVMSYGERPYWEMSNQDVIK
AVDEGYRLPPPMDCPAALYQLMLDCWQKDRNNRPKFEQIVSILDKLIRNPGSLKIITSAAARPSNLLLDQSNVDITTFRTTGDWLNG
VWTAHCKEIFTGVEYSSCDTIAKIS

Vector:

Growth

Medium:
Antibiotics:
Procedure: The protein was expressed in E. coli BL21 (DE3) grown in Terrific Broth (TB) in the presence of 50 microG/mL of kanamycin at 3degºC to an OD600 of 7.5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.05 mM, and incubated overnight at 15degC. The culture was centrifuged and the cell pellets were collected and stored at -80degC.

Purification

Procedure
IMAC purification: 4 microL of clarified supernatant is reserved for later analysis by SDS-PAGE. The rest of the clarified supernatant is then diluted 1:2 in lysis buffer, and loaded at approximately 1mL/min by gravity onto 5 mL of Ni-NTA resin (Qiagen 30450). 5 column volumes of lysis buffer are used to wash the column at approximately 3 mL/min, followed by 5 column volumes of low imidazole buffer (lysis buffer + 10 mM Imidazole (VWR EM-5720) pH 8) at approximately 3 mL/min. A 4 microL sample of the low imidazole wash is saved for later

analysis by SDS-PAGE. Samples are eluted from the Ni-NTA resin by exposure to 10 mL elution buffer (lysis buffer + 250 mM imidazole and 10% glycerol (EMD GX0185-5)) at 1mL/min flow rate. A 10 microL sample of the eluate is saved for SDS-PAGE analysis. 10 microL of each eluate is saved for measurement of protein concentration using Bradford reagent (BioRad 500-0202).

Extraction

Procedure
The cell pellet was resuspended in lysis buffer, inhibitor (0.1 microM phenylmethyl sulfonyl fluoride, PMSF) and lysed using Microfluidizer. The lysate was cleared by centrifugation.
Concentration:
Ligand
MassSpec:
Crystallization: Protein concentration: 20 mg/mL protein
Crystallization conditions: 25% PEG 3350, 0.2M Ammonium Sulfate, 0.1M Hepes, 20% glycerol, pH 7.5, temperature 298K
NMR Spectroscopy:
Data Collection:
Data Processing: