EPHA3

PDB:2QOO

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:epha3.BC063282.OBS.30347891.pBluescript
Entry Clone Source: Open Biosystems
SGC Clone Accession:epha3.0577-0947.102F03
Tag:N-terminal: MGSSHHHHHHSSGLVPRGS
Host:*E. coli* BL21 (DE3)

Construct

Prelude:
Sequence:

MGSSHHHHHHSSGLVPRGSDEKRLHFGNGHLKLPGLRTFVDPHTFEDPTQTVHEFAKELDATNISIDKVVGAGEFGEVCSGRLKLPS
KKEISVAIKTLKVGYTEKQRRDFLGEASIMGQFDHPNIIRLEGVVTKSKPVMIVTEYMENGSLDSFLRKHDAQFTVIQLVGMLRGIA
SGMKYLSDMGFVHRDLAARNILINSNLVCKVSDFGLSRVLEDDPEAAYTTRGGKIPIRWTSPEAIAYRKFTSASDVWSYGIVLWEVM
SYGERPYWEMSNQDVIKAVDEGYRLPPPMDCPAALYQLMLDCWQKDRNNRPKFEQIVSILDKLIRNPGSLKIITSAAARPSNLLLDQ
SNVDITTFRTTGDWLNGVWTAHCKEIFTGVEYSSCDTIAKIS

Vector:pET28a-LIC vector (GenBank, EF442785)

Growth

Medium: Terrific Broth (TB) in the presence of 50 µg/mL of kanamycin
Antibiotics:
Procedure: Using the SGC\'s LEX bubbling system, EPHA3 was expressed in *E. coli* BL21 (DE3) grown in growth medium at 37ºC to an OD_{600} of 7.5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.1 mM and incubated overnight at 15ºC. The culture was centrifuged and the cell pellets were collected and stored at -80ºC.

Purification

Procedure
Lysed cells were diluted to 50-100 mL final volume and imidazole added to a final concentration of 10 mM; this was then mixed with 2-3 mL of HisLink resin (Promega V8821) per construct. The mixture is incubated with mixing for at least 20 minutes at 4 ºC.

IMAC purification: The lysate was spun at 500xg for 3 minutes to pellet the HisLink resin. The lysate was carefully decanted off the resin, and then 50 mL of lysis buffer were added to wash the

resin. The resin was allowed to settle for 5 minutes, then poured off and washed 3 more times with fresh lysis buffer. The washed resin was then loaded onto a gravity column and washed with a column volume of low imidazole buffer. Samples were eluted from the HisLink resin by exposure to 10 mL elution buffer. (His)6-tag cleavage: 1unit of thrombin (Sigma T9681) per milligram of eluted was stored without shaking, overnight, at 4 ºC.

Extraction

Procedure
Frozen cell pellets contained in bags (Beckman 369256) obtained from 2L liters of culture were thawed by soaking in warm water for 5 minutes. Each cell pellet was resuspended in 20 mL lysis buffer and 1 mL Sigma general protease inhibitor (Sigma P2714-1BTL, resuspended according to manufacturerÂ□s instructions) and then 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 with a sonication protocol of 10 sec pulse at half-maximal frequency, 10 second rest, for 6 minutes total sonication time per pellet.
Concentration:20 mg/mL protein
Ligand
MassSpec:
Crystallization:Crystallization conditions: 25% PEG 3350, 0.2M Ammonium Sulfate, 0.1M Hepes, pH 7.5, temperature 298K
NMR Spectroscopy:
Data Collection:
Data Processing: