EPHA2

PDB:3C8X

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_004422.2
Entry Clone Source:epha2.BC037166.MGC.AU80A3.pOTB7
SGC Clone Accession:epha2.023.202.121A05 (SDC121A05)
Tag:N-terminal: APEHHHHHHDYDIPTTENLYFQGAMD
Host: Sf9 insect cells

Construct

Prelude:
Sequence:

apehhhhhhdydipttenlyfqgamdAAQGKEVVLLDFAAAGGELGWLTHPYGKGWDLMQNIMNDMPIYMYSVCNVMSGDQDNWLRT
NWVYRGEAERIFIELKFTVRDCNSFPGGASSCKETFNLYYAESDLDYGTNFQKRLFTKIDTIAPDEITVSSDFEARHVKLNVEERSV
GPLTRKGFYLAFQDIGACVALLSVRVYYKKCP

Vector:pFHMSP-LIC-N

Growth

Medium:
Antibiotics:
Procedure: Plasmid transfer vector pFHMSP-LIC-N containing the gene was transformed into DH10Bac E.coli cells (Invitrogen) to obtain recombinant viral DNA. SF9 cells were transfected with Bacmid DNA using Cellfectin reagent (Invitrogen), and recombinant baculovirus was generated. Viral stock was amplified from P1 to P3.

Sf9 cells grown in HyQ® SFX Insect Serum Free Medium (Cat.# SH3027802) at density of 3.5 million cells per milliliter of media and with viability not less then 97 % were infected with 5 mL of P3 viral stock for each 1 L of cell culture. Cell culture medium was collected after 4 days of incubation on a shaker at 100 RPM and 27 ºC when cells viability dropped to 25-45 %.

Purification

Procedure
IMAC purification: A 3.2 L volume of medium was mixed with 30 ml pre-equilibrated NiNTA Superflow beads and stirred (Talboys/Troemner) for 1 hour. The resin was transferred to a 100 ml gravity column, washed with 300 ml of Washing Buffer, and the protein was eluted with 90 ml of

Elution Buffer. A second round of NiNTA batch absorption may have been performed for increased yield. The eluate was dialyzed against 50-100 X volume of Buffer A overnight at 4 degC.

Extraction

Procedure
The cultured medium was centrifuged at 14,000 xg for 15 minutes, and the pH of the supernatant was adjusted to 7.5 at room temperature by adding 10x Buffer_A. Protease inhibitors were added to final concentrations of 1 mM (phenylmethanesulfonyl fluoride, PMSF, Bioshop) and 2 mM (benzamidine hydrochloride, Sigma).
Concentration: Purified protein was concentrated using 15 mL concentrators with an appropriate molecular weight cut-off (Amicon Ultra-15 10,000 MWCO, Millipore) to a final value of 10 mg/mL. Average yield was about 2 mg/L.
Ligand
MassSpec:
Crystallization: An in-situ proteolysis strategy was used to generate high-quality crystals. Briefly, trypsin was added from a 1.5 g/L stock to a protein sample (at 6.2 g/L) to a final trypsin concentration of 6.2e-3 g/L (1000 X less) before crystal plates were set (500 nL + 500 nL) at 18 ºC. Optimal crystallization conditions centered at 25 % PEG 3350, 0.1 M Ammonium Sulfate, 0.1 M Bis-Tris pH 5.5. Paratone-N was used as cryoprotectant for data-collection at 100 ºK.
NMR Spectroscopy:
Data Collection:
Data Processing: