AXL2

PDB:2P0C

Revision

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Entry Clone Accession:axl2.LIFESEQ1708542.OBS.IMAGE:1708542.pINCY
Entry Clone Source: OpenBiosystems
SGC Clone Accession:SDC037B11
Tag:mgsshhhhhhssglvprgs
Host:E. Coli. BL21(DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsEELQNKLEDVVIDRNLLILGKILGEGEFGSVMEGNLKQEDGTSLKVAVKTMKLDNSSQREIEEFLSEA
ACMKDFSHPNVIRLLGVCIEMSSQGIPKPMVILPFMKYGDLHTYLLYSRLETGPKHIPLQTLLKFMVDIALGMEYLSNRNFLHRDLA
ARNCMLRDDMTVCVADFGLSKKIYSGDYYRQGRIAKMPVKWIAIESLADRVYTSKSDVWAFGVTMWEIATRGMTPYPGVQNHEMYDY
LLHGHRLKQPEDCLDELYEIMYSCWRTDPLDRPTFSVLRLQLEKLLESLPDV

Vector:pET28a-LIC

Growth

Medium:
Antibiotics:
Procedure:A small overnight culture containing 50 µg/mL kanamycin was used to inoculate TB media containing the same concentration of antibiotics. Cultures were grown at 37°C for about 6 hours until the OD600 reached ~0.8, the temperature was adjusted to 15°C, and expression was induced using 0.1mM IPTG overnight. Cells were harvested by centrifugation and frozen.

Purification

Procedure
Clarified supernatant was mixed with 5.0 ml 50% Talon resin slurry (Clonetech), rotated for 1 hour at 4 degrees Celsius, and then loaded into a column. Ten column volumes of lysis buffer plus 10 mM imidazole (VWR EM-5720) were used for washing before elution with 7 mL elution buffer (lysis buffer + 200 mM imidazole). Gel-filtration was conducted using AKTAxpress (18-6645-05, GE Healthcare) with XK 16x65 columns (part numbers 18-1031-47 and 18-6488-01, GE Healthcare) packed with HighLoad Superdex 200 resin (10-1043-04, GE Healthcare). Pre-equilibration was done with gel filtration buffer (20 mM Tris, pH 8.0, 500 mM NaCl, 5%

glycerol, 2 mM β-mercaptoethanol) at a flow rate of 3 mL/min. Seven mL of protein sample was loaded onto the column at 1.5 mL/min, and 2mL fractions are collected in 96-well plates (VWR 40002-012) using peak fractionation protocols with the following parameters: (Slope; min. peak width 0.833 min; level 0.000 mAU; peak start slope 10.000 AU/min; peak end slope 20.000 AU/min). Fractions were analyzed for purity using SDS-PAGE and those containing pure Mer/axl2 were pooled.

Extraction

Procedure
Frozen cell pellets obtained from 2L culture were thawed, resuspended in 50 mL lysis buffer, homogenized (Ultra-Turrax T8 homogenizer (IKA Works) at maximal setting for 30-60 seconds), and twice passed through a microfluidizer (Microfluidics M110EH) at 18,000 psi. The lysate was clarified by centrifugation (JA25.50 rotor, Avanti J-20 XPI, Beckman Coulter) for 20 minutes at 69,673 x g.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals of Mer/axl2 were obtained at 14 degC by hanging drop vapor diffusion with 30 mg/mL protein containing 2.5 mM AMP-PNP and 10 mM MgCl2 mixed with 29% PEG400, 0.2M MgCl2, 0.1 M Tris pH 8.5.
NMR Spectroscopy:
Data Collection: Crystals were cryo-protected with a complex solution composed of glycerol, ethylene glycol, glucose, and fructose. Data was collected on a Rigaku FRE Superbright diffractometer.
Data Processing: