GABARAPL1

PDB:2R2Q
Entry Clone Accession:NP_113600.1
Entry Clone Source:ubh57.BC009309.MGC.AU59F10.pOTB7
SGC Clone Accession:ubh57.003.111; plate SDC112B12
Tag:MHHHHHHSSGRENLYFQG
Host:BL21 (DE3)
Vector:pET28-MHL

Sequence: mhhhhhhssgrenlyfqgFQYKEDHPFEYRKKEGEKIRKKYPDRVPVIVEKAPKARVP DLDKRKYLVPSDLTVGQFYFLIRKRIHLRPEDALFFFVNNTIPPTSATMGQLYEDNHEEDY FLYVAYSD

Growth

Medium:TB
Procedure: A 250 ml flask containing LB (Sigma L7658) supplemented with 50 ug/ ml kanamycin (BioShop Canada KAN 201) was inoculated from a glycerol stock of the bacteria. The flask was shaken overnight (16 hours) at 250 rpm at 37 degC.

Using the Lex system, a 2L bottle (VWR 89000-242) containing 1800 ml of TB (Sigma T0918) supplemented with 1.5% glycerol, 50 ug/ ml kanamycin and 600 ul antifoam 204 (Sigma A-8311) was inoculated with 50 ml overnight LB culture, and incubated at 37 degC. The temperature of the media was reduced to 15 degC one hour prior to induction and induced at $OD_{(600)}$ = 6 with 100 uM isopropyl-thio-ß-D-galactopyranoside (BioShop Canada IPT 001). Cultures were aerated overnight (16 hours) at 15 degC, and cell pellets collected by centrifugation and frozen at -80 degC.

Purification

Procedure:

IMAC: Unclarified lysate was mixed with 2-3 mL of HisLink Protein Purification Resin (Promega V8821) per 40 mL lysate. The mixture was incubated with mixing for at least 20 minutes at 4 degC. The lysate was spun at 500 xg for 5 minutes, and the supernatant was decanted. The remaining resin was washed with 45 mL of cold wash buffer, allowing 5 minutes to settle between washes, until the supernatant was clear (usually 3-5 washes). The washed resin was transferred to a gravity column and further washed with 1 column volumes (approx. 5 mL) of wash buffer at approximately 3 mL/min. Samples were eluted from the resin by exposure to 2-3 column volumes (approx. 10 mL) of elution buffer. The protein was cut overnight at 4 degC after addition of 100U of AcTEV protease (Invitrogen).

Gel filtration chromatography: An XK 16x65 column (GE Healthcare) packed with HighLoad Superdex 200 resin (GE Healthcare) was pre-equilibrated with gel filtration buffer for 1.5 column volumes. The eluate from IMAC (approx. 10 mL) was loaded onto the column at 1.5 mL/min, and 2mL fractions were collected. Peak fractions observed by UV (A280) absorption to contain protein were pooled.

Extraction

Procedure: Frozen cell pellet contained in bags (Beckman) obtained from 2L of culture were thawed by soaking in warm water. Each cell pellet was resuspended in 25 mL lysis buffer and 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: the sonication protocol was 10 sec pulse at half-maximal frequency (5.0), 10 second rest, for 10 minutes total sonication time per pellet.
Concentration: Purified proteins were concentrated using 15 mL concentrators with a 5,000 molecular weight cut-off (Amicon Ultra-15, UFC900524, Millipore) at 3750 rpm, typically resulting in a final concentration of 10-15 mg/mL.

Structure Determination

MassSpec: Mass-spectroscopy by LCMS showed pure product of correct molecular weight.
Crystallization: The protein crystallized under the following condition: 25% PEG 3350, 0.2M Sodium chloride, 0.1M Hepes pH 7.5, 5% MPD, temperature 291K. The protein was mixed 1:1 with reservoir; final drop size was 1 µL in sitting drop format. Crystals were cryoprotected in 20% glycerol before freezing in LN2.