GMPR2

PDB:2BZN

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC008021
Entry Clone Source:MGC
SGC Clone Accession:
Tag:M G S S H H H H H H S S G L V P R G S
Host:BL21(DE3)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsLDFKDVLLRPKRSTLKSRSEVDLTRSFSFRNSKQTYSGVPIIAANMDTVGTFEMAKVLCKFSLFTAVH
KHYSLVQWQEFAGQNPDCLEHLAASSGTGSSDFEQLEQILEAIPQVKYICLDVANGYSEHFVEFVKDVRKRFPQHTIMAGNVVTGEM
VEELILSGADIIKVGIGPGSVCTTRKKTGVGYPQLSAVMECADAAHGLKGHIISDGGCSCPGDVAKAFGAGADFVMLGGMLAGHSES
GGELIERDGKKYKLFYGMSSEMAMKKYAGGVAEYRASEGKTVEVPFKGDVEHTIRDILGGIRSTCTYVGAAKLKELSRRTTFIRVTQ
QVN

Vector:pET28a-LIC

Growth

Medium:
Antibiotics:
Procedure:20 µL competent BL21(DE3) cells were transformed with 1 µL plasmid. 10min on ice, 45sec at 42°C. Added 100µL SOC then 30min at 37°C. Cells were plated on Kanamycin. Colonies were grown in 50mL of TB + 4% glycerol at 37°C, until OD600 of 0.5 then diluted into 400mL TB + 4% glycerol. Growth at 37°C until OD600 of 1.2 - 1.5. Then at 18 °C for 1h. Induction with 0.5mM IPTG at OD600 of 1.4 - 1.8. Left culture at 18 °C over night.

Purification

Procedure
Buffers: 50 mM HEPES, pH 7.5, 10 mM Imidazole, 500 mM NaCl, 10% glycerol, 0.5 mM TCEP (IMAC Bind/Wash1 Buffer); 50 mM HEPES, pH 7.5, 50 mM Imidazole, 500 mM NaCl, 10% glycerol, 0.5 mM TCEP (IMAC Wash2 Buffer); 50 mM HEPES, pH 7.5, 400 mM Imidazole, 500 mM NaCl, 10% glycerol, 0.5 mM TCEP (IMAC Elution Buffer); 20 mM HEPES pH 7.5, 300 mM NaCl, 10% glycerol, 0.5 mM TCEP (Gelfiltration Buffer).

Columns: HisTrap HP 1 mL (IMAC); HiLoadÂ□ 16/60 Superdex 200 Prep Grade (Gel filtration)

Procedure: Purification was conducted automatically on an ÄKTA xpress system operated by UNICORN software at a flow of 0.8 mL/min. Prior to purification columns were equilibrated with IMAC Bind/Wash1 Buffer (HisTrap HP) and Gel filtration buffer (Superdex 75). The protein sample was loaded on the HisTrap HP column that was washed with IMAC Bind/Wash1 Buffer followed by IMAC Wash2 Buffer. Bound protein was eluted from the IMAC columns with 7.5 mL of IMAC Elution Buffer and loaded in the Gel filtration column. The chromatogram from Gel filtration showed one major protein peak that mainly consisted of GMPR2 of high purity as shown by SDS-PAGE analysis. TCEP was added to the pooled protein peak to a final concentration of 2 mM. The protein was concentrated to 15 mg/mL and stored at -80ºC.

Extraction

Procedure
Cells were harvested by centrifugation and pellets were resuspended in 50mM HEPES pH 7.5, 500mM NaCl, 10% glycerol, 50µg/mL of Lysosyme was added as well as 1 tablet Complete EDTA-free protease inhibitor tablet per cell pellet. Cells were disrupted by sonication (60%, 1s-1s pulse on-off) for three minutes. The sample was centrifuged for one hour at 40000×g. The soluble fraction was filtered through 0.22 µm and subjected to further purification.
Concentration:
Ligand
MassSpec:
Crystallization: Purified His-tagged GMPR2 was crystallized using the hanging drop vapour diffusion method with 1µL protein solution (7.5 mg/mL or 3.7 mg/mL) + 1µL reservoir solution. 7.5 mM GMP was added to the protein prior to setting the drops. The drops were equilibrated against a reservoir solution containing 11% PEG3350, 0.1 M Sodium Citrate pH 5.8. Crystals formed after several days.
NMR Spectroscopy:
Data Collection:
Data Processing: