GMPR2 + NADPH/IMP

PDB:2C6Q

Revision

Revision Type:created
Revised by:created
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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:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure:10 mL TB media supplemented with 4 g/L glycerol and 50 µg/mL Kanamycin was inoculated with BL21 DE3 cells. Cells were grown overnight at 30 ºC in 100 mL shake flasks at 200 rpm. 750 mL TB media supplemented with 4 g/L glycerol and 50 µg/mL kanamycin was inoculated with 10 mL of the over night culture. The large scale cultivations were grown in TunAir shake flasks at 37 °C, 225 RPM. When OD reached approximately 1.3. the temperature in the incubator was lowered to 18°C during 30 min. Then the, protein expression was induced by addition of IPTG to a final concentration of 0.5 mM. The protein expression was continued over night.

Purification

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

Procedure:
Purification was conducted automatically on an AKTA 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 200). 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 onto 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, 0.5 mM TCEP and 1 Complete EDTA-free protease inhibitor tablet was added. The pellet was then freezed at -80. Cells were thawed and disrupted by sonication (50%, 2s-2s pulse on-off) for three minutes. The sample was centrifuged for 30 min at 49000×g. The soluble fraction was filtered through 0.22 µm prior to loading onto the column.
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 5 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 9% PEG3350, 0.1 M Sodium Citrate pH 5.5. Crystals formed after several days. The crystal was soaked in 6mM NADPH for 1 hour, cryoprotected in well solution containing 20% glycerol and 6 mM NADPH and then flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection:
Data Processing: