

# GMPR2 + NADPH/IMP

PDB:2C6Q

## 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 S S G L V P R G S

**Host:**BL21(DE3)

## Construct

### Prelude:

#### Sequence:

```
mgsshhhhhssg1vprgsLDFKDVLLRPKRSTLKSRSVDLRSFSFRNSKQTYSGVPIIAANMDTVGTFEMAKVLCKFSLFTAVH  
KHYSLVQWQEFAGQNPDCLEHLAASSGTGSSDFEQLEQILEAIPQVKYICLDVANGYSEHFVEFVKDVRKRFPQHTIMAGNVVTGEM  
VEELILSGADIKVGIGPGSVCTTRKKTGVGYPQLSAVMCADAHGLKGHIISDGGCSCPGDVAKAFGAGADFVMLGGMLAGHSES  
GGELIERDGKKYKLFYGMSEAMMKKYAGGVAEYRASEGKTVEVPFKGDVEHTIRDILGGIRSTCTYVGAAKLKELSRRTTFIRVTQ  
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 °C 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)

HiLoad□ 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 -80°C.

**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 freeze-dried 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 1 µl protein solution (7.5 mg/mL or 5 mg/mL) + 1 µl 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:**