GMPR

PDB:2BLE

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GMPRA-s001
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal His-tag with integrated TEV protease site: mgsshhhhhhssgrenlyfq*gh(m)
Host:E.coli BL21 Rosetta

Construct

Prelude:
Sequence:

mgsshhhhhhssgrenlyfqghmPRIDADLKLDFKDVLLRPKRSSLKSRAEVDLERTFTFRNSKQTYSGIPIIVANMDTVGTFEMAA
VMSQHSMFTAIHKHYSLDDWKLFATNHPECLQNVAVSSGSGQNDLEKMTSILEAVPQVKFICLDVANGYSEHFVEFVKLVRAKFPEH
TIMAGNVVTGEMVEELILSGADIIKVGVGPGSVCTTRTKTGVGYPQLSAVIECADSAHGLKGHIISDGGCTCPGDVAKAFGAGADFV
MLGGMFSGHTECAGEVFERNGRKLKLFYGMSSDTAMNKHAGGVAEYRASEGKTVEVPYKGDVENTILDILGGLRSTCTYVGAAKLKE
LSRRATFIRVTQQHNTVFS

Vector:p11

Growth

Medium:
Antibiotics:
Procedure: The GMPR construct was expressed in E. coli (BL21 Rosetta) in 2 x 1 L Terrific Broth in the presence of 100 µg/mL of ampicillin and 34 µg/mL chloramphenicol at 37oC. Cells were induced at 1 mM IPTG as soon as the OD reached 0.6, and temperature was shifted to 25oC, and culture was grown for 12 hrs. Cells were collected by centrifugation, and pellets were stored frozen (-20 oC) until further use.

Purification

Procedure
HiTrap His column: Lysis buffer: 10mM Imidazole, 300mM NaCl, 50mM NaH_2PO_4. Wash buffer: 20mM Imidazole, 300mM NaCl, 50mM NaH2PO4. Elution Buffer: 250mM Imidazole, 300mM NaCl.

Sample was loaded, wahsed with wash buffer and eluted in elution buffer. The collected peak was injected into size-exclusion chromatography system, and the main peak was selected for

concentration using an Amicon Ultra device.

Superdex S200 column: 10 mM Hepes, pH 7.4, 500 mM NaCl, 5% glycerol

Extraction

Procedure
Pellets were resuspended in 20 mL lysis buffer including Protease inhibitor (complete, Roche), lysed by French Press, and centrifuged to obtained a clear supernatant (15 min, 20.000 x g). Supernatants were processed in a 2 step chromatographic procedure using the Akta xpress (GE Healthcare) purification system.
Concentration:
Ligand
MassSpec:
Crystallization: Diffraction quality crystals with overall dimensions up to 0.6*0.5*0.05 mm were obtained by mixing 600 nl of the protein solution (containing about 7 mM GMP) with 150 nl of the reservoir solution consisting of 0.1 M NaCacodylate pH=6.5, 15% PEG10K, 0.10 M CaAcetate and 25% glycerol. Crystals appeared within a period of several days.
NMR Spectroscopy:
Data Collection:
Data Processing: