# GRM1 + LY341495

**PDB:**3KS9
**Entry Clone Accession:**BC111844
**Entry Clone Source:**Open Biosystems
**SGC Clone Accession:**GRM1:DCC014-E02:C211317
**Tag:**C-terminal tag: EFVEHHHHHHHH
**Host:**Sf9 insect cells
**Vector:**pFHMSP-LIC-C

**Sequence:**

LLAGASSQRSVARMDGDVIIGALFSVHHQPPAEKVPERKCGEIREQYGIQRVEAMFHTLDKINADPVLLPNITLGSEIRDSCWHSSV
ALEQSIEFIRDSLISIRDEKDGINRCLPDGQSLPPGRTKKPIAGVIGPGSSSVAIQVQNLLQLFDIPQIAYSATSIDLSDKTLYKYF
LRVVPSDTLQARAMLDIVKRYNWTYVSAVHTEGNYGESGMDAFKELAAQEGLsIAHSDKIYSNAGEKSFDRLLRKLRERLPKARVVV
CFCEGMTVRGLLSAMRRLGVVGEFSLIGSDGWADRDEVIEGYEVEANGGITIKLQSPEVRSFDDYFLKLRLDTNTRNPWFPEFWQHR
FQCRLPGHLLENPNFKRICTGNESLEENYVQDSKMGFVINAIYAMAHGLQNMHHALCPGHVGLCDAMKPIDGSKLLDFLIKSSFIGV
SGEEVWFDEKGDAPGRYDIMNLQYTEANRYDYVHVGTWHEGVLNIDDYKIQMNKSGLVPRG

## Growth

**Procedure:** Plasmid transfer vector pFHMSP-LIC-C containing the gene was transformed into DH10Bac *E.coli* cells (Invitrogen) to obtain recombinant viral DNA. SF9 cells were transfected with Bacmid DNA using Cellfectin reagent (Invitrogen), and recombinant baculovirus was generated. Viral stock was amplified from P1 to P3.

Sf9 cells grown in HyQ® SFX Insect Serum Free Medium (Cat.# SH3027802) at density of 3 million cells per milliliter of media and with viability not less then 97 % were infected with 7 mL of P3 viral stock for each 1 L of cell culture. Cell culture medium was collected after 4 days of incubation on a shaker at 100 RPM and 27 °C when cells viability dropped to 25-45 %.

## Purification

**Procedure:**

**IMAC purification**: A 4.8 L volume of medium was mixed with 45 mL pre-equilibrated NiNTA Superflow beads and stirred (Talboys/Troemner) for 1 hour. The resin was transferred to a 50 mL gravity column, washed with 600 mL of Washing Buffer1, 240mL of Washing Buffer 2 and the protein was eluted with 30 mL of Elution Buffer. A second round of NiNTA batch absorption has been performed for increased protein yield.

Purified protein was incubated with thrombin (10 U/ml) solution in digestion buffer for 24 hours at 4 degree C then loaded onto the Gelfiltration (GF) column. The chromatogram from gel filtration showed one major protein peak that consisted of GRM1 confirmed by SDS-PAGE analysis.

### Extraction

**Procedure:** The cultured medium was centrifuged at 14,000 xg for 15 minutes, and the pH of the supernatant was adjusted to 7.5 at room temperature by adding 10x Buffer_A. Protease inhibitors were added to final concentrations of 1 mM phenylmethanesulfonyl fluoride (PMSF, Bioshop) and 2 mM benzamidine hydrochloride (Sigma).

**Concentration:**Purified protein was concentrated using 15 mL concentrators with an appropriate molecular weight cut-off (Amicon Ultra-15 50,000 MWCO, Millipore) to a final value of 5 mg/mL. Average yield was about 3 mg/L.

# Structure Determination

**Crystallization:**Crystallization was setup using sitting drops with Red Wings and SGC-I screens initially.
Crystal used for structure determination were grown in: 20% PEG3350, 0.2M KSCN, protein concentration 5mg/mL plus 2mM LY341495.
Cryoprotectant used 0.9V well solution plus 0.1V 80% Glycerol