

GRM7

PDB:3MQ4

Revision

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Entry Clone Accession:NM_000844

Entry Clone Source:OriGeneSGC 25-H7

SGC Clone Accession:GRM7A-s001-C249S:M37-K513

Tag:N-terminal tag: APEHHHHHHHDYDIPTTENLYFQGAMD

Host:Sf9 insect cells

Construct

Prelude:

Sequence:

gamdMYAPHSIRIEGDVTLGGFLFPVHAKGPSGVPCGDIKRENGIHRLEAMLYALDQINSDPNLLPNVTLGARILDTC SRDTYALEQS
LTFVQALIQKDTSDVRCTNGEPPVFVKPEKVVGIVIGASGSSVSIMVANILRLFQIPQISYASTAPELSDDRRYDFFSRVVPPDSFQA
QAMVDIVKALGWNYVSTLASEGSYGEKGVESFTQISKEAGGLsIAQSVRIPQERKDRTIDFDRIIKQLLDTPNsRAVVIFANDEDIK
QILAAAKRADQVGHFLWVGSDSWGSKINPLHQHEDIAEGAITIQPKRATVEGFDAYFTSRTLNNRRNVWFAEYWEENFNCKLTISG
SKKEDTDRKCTGQERIGKDSNYEQEGKVQFVIDAVYAMAHALHHMNKDLCADYRGVCPMEQAGGKKLLKYIRNVNFNGSAGTPVMF
NKNGDAPGRYDIFQYQTTNTSNPGYRLIGQWTDQLNIEDMQWGK

Vector:pFHMSP-LIC-N

Growth

Medium:

Antibiotics:

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. The protein was then TEV cleaved to remove the poly histidine tag. TEV was added in the ration of 50:1 GRM7:TEV. The reaction was incubated at 4°C for ~2 days then loaded onto the Gelfiltration (GF) column. The chromatogram from gel filtration showed one major protein peak that consisted of GRM7 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 3.5 mg/L.

Ligand

MassSpec:

Crystallization: Crystallization was setup using sitting drops with Red Wings and SGC-I screens initially.

Crystal used for structure determination were grown in: 20.0% PEG 3350, 0.2M NaH₂PO₄ protein concentration 5mg/mL plus 2mM LY341495.

Cryoprotectant used: 40.0% PEG 3350, 0.2M NaH₂PO₄ then Paraton.

NMR Spectroscopy:

Data Collection:

Data Processing: