GEMIN5

PDB:5GXH

Revision

Revision Type:created
Revised by:created
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Entry Clone Accession:NM_001252156.1
Entry Clone Source:SGC template CM25-E2
SGC Clone Accession: Gemin5_BV2 (JMC48:E07): M1-A739
Tag:N-terminal tag: MGSSHHHHHHSSGLVPRGS
Host:baculovirus

Construct

Prelude:
Sequence:

MGSSHHHHHHSSGLVPRGSMGQEPRTLPPSPNWYCARCSDAVPGGLFGFAARTSVFLVRVGPGAGESPGTPPFRVIGELVGHTERVS
GFTFSHHPGQYNLCATSSDDGTVKIWDVETKTVVTEHALHQHTISTLHWSPRVKDLIVSGDEKGVVFCYWFNRNDSQHLFIEPRTIF
CLTCSPHHEDLVAIGYKDGIVVIIDISKKGEVIHRLRGHDDEIHSIAWCPLPGEDCLSINQEETSEEAEITNGNAVAQAPVTKGCYL
ATGSKDQTIRIWSCSRGRGVMILKLPFLKRRGGGIDPTVKERLWLTLHWPSNQPTQLVSSCFGGELLQWDLTQSWRRKYTLFSASSE
GQNHSRIVFNLCPLQTEDDKQLLLSTSMDRDVKCWDIATLECSWTLPSLGGFAYSLAFSSVDIGSLAIGVGDGMIRVWNTLSIKNNY
DVKNFWQGVKSKVTALCWHPTKEGCLAFGTDDGKVGLYDTYSNKPPQISSTYHKKTVYTLAWGPPVPPMSLGGEGDRPSLALYSCGG
EGIVLQHNPWKLSGEAFDINKLIRDTNSIKYKLPVHTEISWKADGKIMALGNEDGSIEIFQIPNLKLICTIQQHHKLVNTISWHHEH
GSQPELSYLMASGSNNAVIYVHNLKTVIESSPESPVTITEPYRTLSGHTAKITSVAWSPHHDGRLVSASYDGTAQVWDALREEPLCN
FRGHQGRLLCVAWSPLDPDCIYSGADDFCVHKWLTSMQDHSRPPQGKKSIELEKKRLSQPKA

Vector:pFBOH-Lic

Growth

Medium:
Antibiotics:
Procedure: Baculovirus P1, P2, P3

Purification

Buffers
Wash buffer: 20 mM Tris pH 7.5, 400 mM NaCl, 30 mM imidazole - Elution buffer: 20 mM Tris pH 7.5, 400 mM NaCl, 500 mM imidazole - Gel filtration buffer: 10 mM Tris, pH 7.5, 150 mM NaCl, 1 mM DTT
Procedure
IMAC: Unclarified lysate was mixed with 2-3 mL of Ni-NTA superflow Resin (Qiagen) per 40 mL lysate. The mixture was incubated with mixing for at least 45 minutes at 4oC. The mixture

was then loaded onto an empty comLum (BioRad) and washed with 100 mL wash buffer. Samples were eluted from the resin by exposure to 2-3 column volumes (approx. 10-15 mL) of elution buffer. Concentration of eluted protein was estimated by OD280. Gel filtration chromatography: An XK 26x65 column (GE Healthcare) packed with HighLoad Superdex 75 resin (GE Healthcare) was pre-equilibrated with gel filtration buffer for 1.5 column volumes using an AKTA explorer (GE Healthcare) at a flow rate of 1.0 mL/min. The dialyzed sample from the IMAC step (approx. 15 mL) was loaded onto the column at 1.5 mL/min, and 2mL fractions were collected into 96-well plates (VWR 40002-012) using peak fractionation protocols). Fractions observed by a UV absorption chromatogram to contain the protein were pooled.

Extraction

Buffers
Lysis buffer: 20 mM Tris pH 7.5, 400 mM NaCl, 0.5 mM phenylmethane-sulfonyl fluoride
Procedure
Frozen cells from 6L insecect cell culture were thawed and resuspended in 450 mL extraction buffer supplemented with 0.5 % 0.1M PMSF,and 3 uL benzonase (Sigma Catalog # E1014, 250U/uL), and lysed using sonication for 10 min at 100 W, 10 sec on/10 sec off duty cycle.
Concentration: Purified proteins were concentrated using 15 mL concentrators with a 10,000 molecular weight cut-off (Amicon Ultra-15, UFC900524, Millipore) at 3750 rpm, typically resulting in a final concentration around 20 mg/mL.
Ligand
MassSpec:
Crystallization: The Gemin5 WD40 domain (15 mg /ml) was mixed with the synthesized RNA AAUUUUUG in a ratio of 1:1.5-1:2 and put on ice for 30 minutes before crystallization. The complex was crystallized in a buffer containing 0.1 M Na-Citrate, pH 5.5, 0.2 M Ammonium Acetate, and 15% PEG4K.The crystals were soaked in a cryoprotectant consisting of 85 to 90% reservoir solution and 10 to 15% glycerol (v/v).
NMR Spectroscopy:
Data Collection:
Data Processing: