# GCN5L2

**PDB:**3D7C

## Revision

**Revision Type:**created
**Revised by:**created
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**Entry Clone Accession:**IMAGE:5575574
**Entry Clone Source:**MGC
**SGC Clone Accession:**
**Tag:**Tag sequence: mhhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*) N-terminal his6 tag.
**Host:**BL21(DE3)-R3-pRARE2

## Construct

**Prelude:**
**Sequence:**

mhhhhhhssgvdlgtenlyfq*sMELKDPDQLYTTLKNLLAQIKSHPSAWPFMEPVKKSEAPDYYEVIRFPIDLKTMTERLRSRYYV
TRKLFVADLQRVIANCREYNPPDSEYCRCASALEKFFYFKLKEGGLIDK

**Vector:**pNIC28-Bsa4

## Growth

**Medium:**
**Antibiotics:**
**Procedure:**Growth medium, induction protocol: 1ml from a 10 ml overnight culture containing 50 µg/ml kanamycin and 35 µg/ml chloramphenicol was used to inoculate 1 liter of LB media containing the same concentration of antibiotics. Cultures were grown at 37°C until the OD600 reached ~0.3. After that the temperature was adjusted to 20°C. Expression was induced over night using 1mM IPTG at an OD600 of 0.8.The cells were collected by centrifugation and the pellet resuspended in binding buffer and frozen. Binding buffer: 50mM HEPES pH 7.5; 500 mM NaCl; 5% glycerol, 10 mM imidazole.

## Purification

**Procedure**
Column 1: Ni-affinity chromatography.Buffers: Binding buffer: Binding buffer: 50 mM HEPES pH 7.5, 500 mM NaCl , 5% Glycero, 10mM Imidazole. Wash buffer: 50 mM HEPES pH 7.5, 500 mM NaCl , 30 mM Imidazole , 5% glycerol. Elution buffer: 50 mM HEPES pH 7.5, 500 mM NaCl , 50 to 250 mM imidazole, 5% Glycerol.Procedure: 5 ml of 50% Ni-NTA slurry (Qiagen) was applied to a 1.5 x 10 cm gravity column. The column was equilibrated with 50 ml binding buffer. The lysate was applied to the column which was subsequently washed with 50 ml wash buffer 1 and 2. The protein was eluted by gravity flow by applying 5 ml portions of elution buffer with increasing concentration of imidazole (50 mM, 100 mM, 150mM, 250 mM); fractions were collected until essentially all protein was eluted. The eluted protein was analyzed by SDS-PAGE. DTT was added to the protein sample to a final concentration of 5mM.Column 2: Size exclusion chromatography (Superdex S75, 60 x 1cm)SEC-Buffers: 50 mM Hepes, pH 7.5, 150 mM NaCl, 5 mM DTT.Procedure: The fractions eluted of the Ni-affinity chromatography were concentrated to about 4 mls using Centricon concentrators (5 kDa cut off). The concentrated protein was applied to a Superdex S75 column equilibrated in SEC buffer at a flow rate of 0.8 ml/min. Eluted fractions were 95% pure as judged by SDS-PAGE.

## Extraction

**Procedure**
Extraction method: Cell pellets were lysed by C5 high pressure homogenizer (Avestin). The lysate was centrifuged at 21,000 rpm for 60 minutes and the supernatant collected for purification.
**Concentration:**Protein concentration: Centricon with a 5 kDa cut off in SEC-buffer.
**Ligand**
**MassSpec:**Mass spec characterization: The mass of the protein was calculated to be 15975 Da and experimentally determined mass was 15975 Da for the His tag containing protein.
**Crystallization:**Crystallization: Crystals were obtained using the vapor diffusion method. The protein was concentrated in gel filtration buffer to a protein concentration of 8.2 mg/ml. Sitting drops comprising 100 nl of the concentrated protein mixed with 50 nl of a well solution (0.1M TRIS pH 8.5, 25% PEG 3350) were equilibrated against well solution at 4°C. Crystals appeared within 2-3 days.
**NMR Spectroscopy:**
**Data Collection:**Data Collection: Crystals were cryo-protected using the well solution supplemented with 25% Ethylene Glycol and flash frozen in liquid nitrogen. Diffraction data were collected from a single crystal on a Rigaku FR-E SuperBright at a single wavelength of 1.5 Å. The structure was solved by molecular replacement and refined to 2.06 Å.
**Data Processing:**