NUP43

PDB:4I79
Entry Clone Accession:NP_942590.1
Entry Clone Source:MGC AT85-H7 (BC065028)
SGC Clone Accession:NUP43_BV1 (JMC015:D03): M1-S380
Tag:N-terminal tag: MGSSHHHHHHSSGLVPLGS
Host:baculovirus
Vector:pFBOH-Lic

Sequence:

MGSSHHHHHHSSGLVPRGSMEEIYAKFVSQKISKTRWRPLPPGSLQTAETFATGSWDNEENYISLWSIGDFGNLDSDGGFEGDHQLL
CDIRHHGDVMDLQFFDQERIVAASSTGCVTVFLHHPNNQTLSVNQQWTTAHYHTGPGSPSYSSAPCTGVVCNNPEIVTVGEDGRINL
FRADHKEAVRTIDNADSSTLHAVTFLRTPEILTVNSIGQLKIWDFRQQGNEPSQILSLTGDRVPLHCVDRHPNQQHVVATGGQDGML
SIWDVRQGTMPVSLLKAHEAEMWEVHFHPSNPEHLFTCSEDGSLWHWDASTDVPEKSSLFHQGGRSSTFLSHSISNQANVHQSVISS
WLSTDPAKDRIEITSLLPSRSLSVNTLDVLGPCLVCGTDAEAIYVTRHLFS

Growth

Procedure: Baculovirus P1, P2, P3

Purification

Procedure:

IMAC: Unclarified lysate was mixed with 1-2 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

Procedure: Frozen cell pellet contained in bags (Beckman 369256) obtained from 6L of culture were thawed by soaking in warm water. Each cell pellet was resuspended in 25-40 mL lysis buffer and homogenized using an Ultra-Turrax T8 homogenizer (IKA Works) at maximal setting for 30-60 seconds per pellet. Cell lysis was accomplished by sonication (Virtis408912, Virsonic) on ice: the sonication protocol was 10 sec pulse at half-maximal frequency (5.0), 10 second rest, for 10 minutes total sonication time per pellet.
Concentration: Purified proteins were concentrated using 15 mL concentrators with a 5,000 molecular weight cut-off (Amicon Ultra-15, UFC900524, Millipore) at 3750 rpm, typically resulting in a final concentration around 20 mg/mL.

Structure Determination

Crystallization: Recombinant human NUP43 WD40 repeats was crystallized using the hanging dropvapour diffusion method at 18 °C. The crystals were obtained in a buffer containing 0.1 M Sodium Hepes, pH 7.5, 0.1 M Ammonium Sulphate, 25 % PEG 3350. Crystals were soaked in a cryoprotectant consisting of 100% reservoir solution and 12% glycerol.