LOC148158

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Revision

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Entry Clone Accession:gi|32699052
Entry Clone Source: Origene
SGC Clone Accession:NM_182617 Variant
Tag:N-terminal, TEV cleavable hexahistidine tag. Tag sequence: mhhhhhhssgvdlgtenlyfq(*)sm
Host: Trichoplusia Ni (High five)

Construct

Prelude:
Sequence:

mghhhhhhssgvdlgtenlyfq*sMSLQWGHQEVPAKFNFASDVLDHWADMEKAGKRPPSPALWWVNGKGKELMWNFRELSENSQQA
ANVLSGACGLQRGDRVAVVLPRVPEWWLVILGCIRAGLIFMPGTIQMKSTDILYRLQMSKAKAIVAGDEVIQEVDTVASECPSLRIK
LLVSEKSCDGWLNFKKLLNEASTTHHCVETGSQEASAIYFTSGTSGLPKMAEHSYSSLGLKAKMDAGWTGLQASDIMWTISDTGWIL
NILCSLMEPWALGACTFVHLLPKFDPLVILKTLSSYPIKSMMGAPIVYRMLLQQDLSSYKFPHLQNCVTVGESLLPETLENWRAQTG
LDIRESYGQTETGLTCMVSKTMKIKPGYMGTAASCYDVQIIDDKGNVLPPGTEGDIGIRVKPIRPIGIFSGYVDNPDKTAANIRGDF
WLLGDRGIKDEDGYFQFMGRADDIINSSGYRIGPSEVENALMEHPAVVETAVISSPDPVRGEVVKAFVVLASQFLSHDPEQLTKELQ
QHVKSVTAPYKYPRKIEFVLNLPKTVTGKIQRAKLRDKEWKMSGKARAQ

Vector: pFB-LIC-Bse

Growth

Medium:
Antibiotics:
Procedure: High five cells were grown in Sf900II medium supplemented with 1% FCS at 27°C. Cells were infected at a density of 2x106/ml with recombinant baculovirus (virus stock P2; 1ml of virus stock/1l of cell culture). 72 hours post-infection the cultures were collected and centrifuged for 30min at 2000rpm. The cell pellet was resuspended in cold PBS and centrifugation was repeated. The pellet was resuspended in lysis buffer (50mM HEPES pH 7.5, 500mM NaCl, 5mM Imidazole, 5% glycerol + EDTA-free Complete (1 tablet/50ml)) and frozen at -80°C until purification.

Purification

Procedure
Step 1: Ni-affinity, Ni-Sepharose - (GE Healthcare) purification in batchStep 2: Superdex 200Column , HiPrep 16/60 (Amersham)Step 3: Ion exchange -5ml HiTrap Q SepharoseThe cell

extract was incubated with Ni-Sepharose (0.250µl of resin/lysate from 1l of culture) during 1h at 4°C with gentle rotation. The resin was centrifuged at 1000g for 5min at 4°C, and washed 4x with 50ml of washing buffer. Resin was loaded on the gravity column and protein was eluted in 4 elution fractions (5ml each). Protein fractions were analysed by SDS-PAGE. Target protein containing fractions were concentrated using Amicon Ultra-15 concentrators with 10kDa cut-off, and purified on a gel filtration column (Superdex 200) on an Akta Purifier System. LOC148158 containing fractions were diluted with IEX buffer A to a concentration of NaCl of 50 mM. The protein was loaded onto a HiTrap Q Sepharose column, and eluted with a NaCl gradient (50-300mM). Fractions containing protein were analysed by SDS-PAGE.

Extraction

Procedure
The thawed cellular lysates were supplemented with Igepal CA 630 (Fluka) at a final concentration of 0.05%, and benzonase (Novagen) (25u/10ml of lysate). Cells were broken using a Dounce homogeniser, followed by centrifugation for 45 min at 20.000rpm at 4°C.
Concentration: Using Amicon Ultra-15 concentrators with 10kDa cutoff, the sample was concentrated to 20mg/ml. Concentrations were determined from the absorbance at 280 nm using a NanoDrop spectrophotometer.
Ligand
MassSpec: The calculated mass of the construct was 63260Da, and the observed mass (ESI-MS) was 63173Da, compatible with an N-terminal methionine deletion and subsequent acetylation at the N-teminus of the protein.
Crystallization: Crystals were grown by vapor diffusion at 20°C in 150nl sitting drops. Acetyl-CoA was added to a final concentration of 1mM prior to crystallisation. The drops were prepared by mixing 100nl of protein solution and 50nl of precipitant consisting of 0.1M HEPES pH 7.5; 1.4M Na-citrate. Crystals were flash-cooled in liquid nitrogen with 25 % glycerol as cryoprotectant.
NMR Spectroscopy:
Data Collection: Resolution: 2.0Å; X-ray source: Swiss Light source (SLS), beamline X-10.
Data Processing: