HUWE1

PDB:3G1N
Entry Clone Accession:NP_113584
Entry Clone Source:huwe1.NM_031407.LAB.Martin_Eilers_dnd.pCMVHA
SGC Clone Accession:huwe1.4006.4374.158A03 (SDC158A03)
Tag:N-terminal: MHHHHHHSSGRENLYFQG
Host:BL-21(DE3)V2R
Vector:pET28-MHL

Sequence:

mhhhhhhssgrenlyfqgLERLDEGLRKEDMAVHVRRDHVFEDSYRELHRKSPEEMKNRLYIVFEGEEGQDAGGLLREWYMIISREM
FNPMYALFRTSPGDRVTYTINPSSHCNPNHLSYFKFVGRIVAKAVYDNRLLECYFTRSFYKHILGKSVRYTDMESEDYHFYQGLVYL
LENDVSTLGYDLTFSTEVQEFGVCEVRDLKPNGANILVTEENKKEYVHLVCQMRMTGAIRKQLAAFLEGFYEIIPKRLISIFTEQEL
ELLISGLPTIDIDDLKSNTEYHKYQSNSIQIQWFWRALRSFDQADRAKFLQFVTGTSKVPLQGFAALEGMNGIQKFQIHRDDRSTDR
LPSAHTCFNQLDLPAYESFEKLRHMLLLAIQECSEGFGLA

Growth

Medium:TB

Procedure: A volume of 1.8 L of TB (Sigma T0918) supplemented with glycerol (final conc 100 µM), Kanamycin (final conc 85 µM), and antifoam 204 (Sigma A-8311; about 600 µL) was inoculated with 50 mL of overnight LB pre-culture and aerated with the LEX system at 37 °C. When the OD600 6, the temperature of the media was reduced to 15 °C (which required about 1 hour) and the culture was induced with 100 µM IPGT (BioShopCanada IPT001). Cultures were aerated overnight (16 hours) at 15 °C, and cell pellets were collected by centrifugation and frozen in liquid nitrogen before storage.

Purification

Procedure: Cleared lysate was rocked with TALON metal-affinity resin (BD Biosciences; 1.5 mL settled beads per L cell culture) at 4 °C. The column was washed with 5 column volumes of Wash buffer A, 5 column volumes of Wash buffer B, and 5 column volumes of Wash buffer A. The protein was eluted with 2 column volumes of Elution buffer.

The protein was further purified by gel filtration through a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with Gel Filtration buffer. Fractions containing protein (analyzed by ABS280 nm) were pooled and concentrated by ultra filtration using an Amicon Ultra centrifugal filter with 5 kDa cutoff.

The yield of the protein was approximately 2 mg per L of bacterial culture.

Extraction

Procedure: Cell pellets were resuspended in Lysis buffer (30 mL per L culture), lysed using a Microfluidizer (18,000 psi), and cleared by centrifugation (40,000 xg for 30 minutes).

Structure Determination

MassSpec: Mass-spectroscopy by LCMS showed that the product was pure and had the correct molecular weight .
Crystallization: Crystals were grown at 20 °C using the hanging drop method by mixing 2 volumes of the protein (20 mg/mL in 20 mM TrispH8.0, 300 mM NaCl, 0.5 mM TCEP) with 1 volume of well solution consisting of 15% PEG-4000, 10% iso-propanol, 0.1 M Tris-pH7.5. Crystals were cryoprotected by emersion in well solution supplemented with 50% (volume/volume) paratone before frozen in liquid nitrogen.