HECTD1

PDB:3DKM
Entry Clone Accession:NP_056197
Entry Clone Source:hectd1.BAA86445.KAZUSA.KIAA1131.pBluescriptSK+
SGC Clone Accession:hectd1.1273.1343.094B07 (SDC94B07)
Tag:N-terminal: MHHHHHHSSGRENLYFQG
Host:BL21 (DE3)
Vector:pET28-MHL

Sequence:

mhhhhhhssgrenlyfqgLKYMVPGARVTRGLDWKWRDQDGSPQGEGTVTGELHNGWIDVTWDAGGSNSYRMGAEGKFDLKLAPGYD
PD

Growth

Procedure: A 250 ml flask containing LB (Sigma L7658) supplemented with 50 ug/ ml kanamycin (BioShop Canada KAN 201) was inoculated from a glycerol stock of the bacteria. The flask was shaken overnight (16 hours) at 250 rpm at 37 degC.

Using the Lex system, a 2L bottle (VWR 89000-242) containing 1800 ml of TB (Sigma T0918) supplemented with 1.5% glycerol, 50 ug/ ml kanamycin and 600 ul antifoam 204 (Sigma A-8311) was inoculated with 50 ml overnight LB culture, and incubated at 37 degC. The temperature of the media was reduced to 15 degC one hour prior to induction and induced at OD600 = 6 with 100 uM isopropyl-thio-ß-D-galactopyranoside (BioShop Canada IPT 001). Cultures were aerated overnight (16 hours) at 15 degC, and cell pellets collected by centrifugation and frozen at -80 degC.

Purification

Procedure:

Cleared lysate was loaded onto TALON metal-affinity resin column (BD Biosciences; 1.5 ml

settled beads per L cell culture) at 4ºC. The column was washed with 5 column volumes (cv) of Wash buffer A, 5 cv Wash buffer B, and 5 cv Wash buffer A. The protein was eluted with 2 cv Elution buffer.

The protein was further purified by gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare, Amersham) equilibrated with Gel Filtration buffer. Fractions containing protein (analyzed by SDS-PAGE) were pooled and concentrated by ultrafiltration using an Amicon Ultra centrifugal filter with 5 kD cutoff to a final concentration of 20 mg/ml. The yield of the protein was approximately 6 mg per liter of bacterial culture.

Extraction

Procedure:
Cell pellets were resuspended in Lysis buffer (30 ml/L culture), lysed using a Microfluidizer (18,000 PSI), and cleared by centrifugation (40,000 x g for 30 min).
Concentration:16 mg/ml

Structure Determination

MassSpec: Mass-spectroscopy by LCMS shows that the product was pure and of correct molecular weight
Crystallization: Crystals of the hectd1 mib-herc2 domain were grown at 298 K using the sitting drop method by mixing 1 volume of 16 mg/ml protein with 1 volume of well solution consisting of 20% PEG 5K, 0.1 M Bis-tris buffer, pH 6.5. The crystals were cryoprotected by immersion in the well solution supplemented with 25% (v/v) glycerol.