HERC1

PDB:4O2W
Entry Clone Accession:SGC cDNA collection 37-F4
Entry Clone Source: Kazusa:ORK07572
SGC Clone Accession:YTC013-A06
Tag:N-terminal His6-tag, not removed
Host:BL21(DE3)-V2R
Vector:pET28-MHL
Prelude:HERC1:G3975-A4360

Sequence: mhhhhhhssgrenlyfqgGMDEQIMSWATSRPEDWHLGGKCDVYLWGAGRHGQLA
EAGRNVMVPAAAPSFSQAQQVICGQNCTFVIQANGTVLACGEGSYGRLGQGNSDDLHV
LTVISALQGFVVTQLVTSCGSDGHSMALTESGEVFSWGDGDYGKLGHGNSDRQRRPRQI
EALQGEEVVQMSCGFKHSAVVTSDGKLFTFGNGDYGRLGLGNTSNKKLPERVTALEGY
QIGQVACGLNHTLAVSADGSMVWAFGDGDYGKLGLGNSTAKSSPQKIDVLCGIGIKKVA
CGTQFSVALTKDGHVYTFGQDRLIGLPEGRARNHNRPQQIPVLAGVIIEDVAVGAEHTLA
LASNGDVYAWGSNSEGQLGLGHTNHVREPTLVTGLQGKNVRQISAGRCHSAAWTA

Growth

Procedure: LEX Bubbling. The target protein was expressed in E. coli by inoculating 30 mL of overnight culture grown in Luria-Bertani medium into a 2 L of Terrific Broth medium in the presence of 50 ug/mL kanamycin at 37 degree. When OD600 reached ~2.5, the temperature of the medium was lowered to 18 degree and the culture was induced with 1.0 mM IPTG. The cells were allowed to grow overnight before harvested by centrifugation (7,000 rpm 15min) and flash frozen in liquid nitrogen and stored at -80 degree.

Purification

Procedure: The lysate was centrifuged at 16,000 rpm (25,800xg RCF(average) for 60 minutes and the supernatants were loaded onto 5 mL Nickel metal-affinity resin column. The column was then washed 3 times with 15 mL washing buffer. Bound proteins were eluted using 10 mL elution buffers A and B. Pooled fractions were combined and were further purified using Superdex 200 column pre-equilibrated with gel filtration buffer. Fractions containing the target protein were pooled and concentrated using Amicon Ultra-15 centrifugal filter (mwco 10 kDa) to a final concentration of about 30 mg/mL. The purity of the preparation is tested by SDS-PAGE and >95%.

Extraction

Procedure: 4L cell pellet was resuspended in a total volume of 200 ml lysis buffer and the cells disrupted by sonication.
Concentration: 30.0 mg/mL, concentration used for crystallization 15.0 mg/mL

Structure Determination

MassSpec: Uncut version native protein expected 42875.9, measured 42876.4.
Crystallization: Initial hits were seen from conditions Red Wings G07 and SGC-II A10. After optimization the pH of the buffer, different PEG (PEG1500, PEG3350, PEG8000) and the ratio of

protein to buffer, the third RLD domain of HERC1 was crystallized by vapor diffusion at 18°C from a hanging drop consisting of 2.0ul protein (15.0 mg/ml) and 1.0ul well solution containing 20% PEG1500, 0.2M MgCl2, 0.1M HEPES pH 7.5. The crystal was transferred to a cryo protectant containing 15% ethylene glycerol in the well solution before flash-frozen in liquid nitrogen