HERC2

PDB:4L1M
Entry Clone Accession:SGC cDNA collection 10-D2: BC045544
Entry Clone Source: Open Biosystems
SGC Clone Accession:YTC007-A11
Tag:N-terminal His6-tag, not removed
Host:BL21(DE3)-V2R
Vector:pET28-MHL
Prelude:HERC2:S417-F790

Sequence:

mhhhhhhssgrenlyfqgSHKGSLQEVIGWGLIGWKYYANVIGPIQCEGLANLGVTQIACAEKRFLILSRNGRVYTQAYNSDTLAPQ
LVQGLASRNIVKIAAHSDGHHYLALAATGEVYSWGCGDGGRLGHGDTVPLEEPKVISAFSGKQAGKHVVHIACGSTYSAAITAEGEL
YTWGRGNYGRLGHGSSEDEAIPMLVAGLKGLKVIDVACGSGDAQTLAVTENGQVWSWGDGDYGKLGRGGSDGCKTPKLIEKLQDLDV
VKVRCGSQFSIALTKDGQVYSWGKGDNQRLGHGTEEHVRYPKLLEGLQGKKVIDVAAGSTHCLALTEDSEVHSWGSNDQCQHFDTLR
VTKPEPAALPGLDTKHIVGIACGPAQSFAWSSCSEWSIGLRVPF

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 10 mL Nickel metal-affinity resin column. The column was then washed with 30 mL of washing buffer 3 times. Bound proteins were eluted using 15 mL elution buffers of A and B. Pooled fractions were combined and were further purified using Superdex 200, 26/60 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 40 mg/mL. The purity of the preparation was tested by SDS-PAGE and were better than 95%.

Extraction

Procedure: 4L cell pellet was resuspended in a total volume of 200 ml lysis buffer and the cells disrupted by sonication

Concentration:40.0 mg/mL, concentration used for the crystal optimization was 30.0 mg/mL.

Structure Determination

MassSpec: Uncut version native protein expected 42006.3, measured 42006.7.
Crystallization: Initial hits were obtained from conditions Red Wings C1 and SGC-II F4. The conditions were optimized using a serial dilution. The first RLD domain of HERC2 was crystallized by vapor diffusion method at 18 degC from a hanging drop consisting of 1.5ul protein (30.0 mg/ml) and 1.5ul well solution containing 1.5M (NH4)2SO4, 0.1 M Tris-HCl at pH 8.5. The crystal was transferred to a cryo protectant containing 16% glycerol in the well solution before flash-frozen in liquid nitrogen.