HMCES

PDB:5KO9

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC010125
Entry Clone Source:MGC AU62-F2
SGC Clone Accession:PBC006-B11
Tag:C-terminal His6-tag, removed
Host:BL21(DE3)V2R-pRARE2

Construct

Prelude:
Sequence:

```
CGRTSCHLPRDVLTRACAYQDRRGQQRLPEWRDPDKYCPSYNKSPQSNSPVLLSRLHFEKDADSSERIIAPMRWGLVPSWFKESDPS
KLQFNTTNCRSDTVMEKRSFKVPLGKGRRCVVLADGFYEWQRCQGTNQRQPYFIYFPQIKTEKSGSIGAADSPENWEKVWDNWRLLT
MAGIFDCWEPPEGGDVLYSYTIITVDSCKGLSDIHHRMPAILDGEEAVSKWLDFGEVSTQEALKLIHPTENITFHAVSSVVNNSRNN
TPECLAPVaenlyfq DNA sequence has been verified by sequencing
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Vector:pNIC-CH

Growth

Medium:
Antibiotics:
Procedure:LEX Bubbling - For native protein: The target protein was over-expressed in E. coli at 37degree C by inoculating 20 mL of overnight culture grown in Luria-Bertani medium into 2 L TerrificBroth medium in the presence of 40 ug/mL kanamycin and 25 ug/mL chloramphenicol. When the OD600 of the culture reached ~1.5, the temperature was lowered to 18 degree and the culture was induced with 0.25 mM final IPTG concentration. The cells were allowed to grow overnight before harvested by centrifugation (5,000 rpm Beckman JLA-8.1000rotor 15 min) and flash frozen in liquid nitrogen and stored at -80 degree.

Purification

Buffers
Washing Buffer: 50 mM Tris-HCl pH8.0, 500 mM NaCl, 50 mM imidazole - Elution Buffer: 50 mM Tis-HCl pH8.0, 500 mM NaCl, 5% Glycerol, 250 mM imidazole - Dialysis Buffer: 50 mM Tris-HCl pH8.0, 300 mM NaCl, 2 mM TCEP - Ion Exchange Buffer A(B): 20 mM Tris-HCl pH 8.0, 0(1) M NaCl, 1 mM TCEP - Gel Filtration Buffer: 20 mM Tris-HCl pH 8.0, 150 mM NaCl, 1

mM TCEP
Procedure
The lysate was centrifuged at 15,000 rpm for 30minutes. The supernant was supplemented with 4 mL Ni-NTA resin (50% flurry) and incubated on a rotary drum for 1 hour at 4 degree, then loaded onto Bio-rad gravity column.The beads deposited in the open column was then washed with 20CV lysis buffer followed by 10CV washing buffer. Bound proteins were eluted using 5CV elution buffer. The N-terminal His-tag was reomoved by overnight incubation with TEV protease (1:30 w/w) at 4degree during dialysis against the dialysis buffer. Uncut proteins and TEVprotease were removed by passing the solution through 1mL Ni-NTA beads,andthe target protein was further purified by anion-exchange chromatography on a 5mL HiTrap Q column(GE Healthcare). The protein was further purified using gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare)preequilibrated with gel filtration buffer. Fractions containing the targetprotein were pooled and concentrated by centrifugal filters (Amicon mwco10kDa).The yields of the protein was about 4.2 mg per litre bacterialculture with >95% purity.

Extraction

Buffers
50 mM Tris-HCl pH 8.0, 500 mM NaCl, 5% Glycerol
Procedure
2L native cell pellet was resuspended in a total volume of 200 ml extraction buffer with1mM PMSF/Benzamidine freshly added and the cells disrupted by sonication for 10 mins at 5" on 10" off duty cycle at 108W output power.
Concentration: Concentration used for crystallization : 18.0 mg/mL
Ligand
MassSpec:measured (after His-tag cleavage): 31.632.9 Da
Crystallization: Crystals used for structure determination were grown at 293K in sitting drop Vapor Diffusion by mixing 1 uL protein solution with 1 uL well solution containing 0.1M BTP, 2% Tacsimate, 20% PEG3350, pH 6.5.The crystals were cryoprotected by 30% Glycerol before flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection:
Data Processing: