Entry Clone Source: MGC

Entry Clone Accession: IMAGE:4478358

SGC Construct ID: HDHD1AA-c000

GenBank GI number: gi|50726983

Vector: pNIC28-Bsa4. Details [PDF]; Sequence [FASTA] or [GenBank]

Coding DNA sequence:

```
CATATGCACCATCATCATCATCATTCTTC
TGGTGTAGATCTGGGTACCGAGAACCTGT
ACTTCCAATCCATGGCGGCGCCCCCGCAG
CCCGTCACCCACCTCATCTTTGACATGGA
CGGACTTCTTCTGGATACTGAACGGCTGT
ATTCAGTGGTGTTTCAAGAAATATGTAAT
CGCTATGACAAGAAATACAGCTGGGATGT
AAAGTCCCTGGTTATGGGTAAGAAGGCAT
TAGAGGCGGCACAGATTATAATAGACGTC
TTGCAGCTCCCGATGTCCAAAGAGGAGCT
GGTGGAAGAAAGCCAAACGAAGTTAAAGG
AAGTGTTCCCCACGGCTGCGCTCATGCCA
GGGGCGGAGAAACTCATCATCCACCTGCG
GAAACATGGCATCCCCTTTGCACTGGCCA
CCAGCTCGAGGTCCGCGTCGTTCGATATG
AAGACAAGCCGCCACAAGGAGTTCTTCAG
CTTGTTTTCCCACATTGTGCTGGGAGATG
ACCCCGAAGTGCAGCATGGCAAGCCAGAC
CCAGACATCTTCCTAGCTTGTGCCAAGAG
GTTCTCTCCCCCTCCTGCTATGGAGAAGT
GCCTTGTCTTTGAAGATGCTCCCAATGGG
GTGGAGGCGGCCCTGGCAGCTGGGATGCA
GGTGGTCATGGTTCCTGACGGAAACTTGA
GCCGAGATCTGACAACAAAGGCCACCCTG
GTGCTGAATTCCCTGCAGGACTTCCAGCC
CGAGCTGTTTGGTTTGCCCTCCTATGAGT
GACAGTAAAGGTGGATACGGATCCGAA
```

Tags and additions: N-terminal TEV-cleavable (at *) his-tag with the following sequence:
mhhhhhhssgvdlgtenlyfq*s

Host: BL21(DE3)-R3

Protein sequence (tag sequence in lowercase):

```
mhhhhhhssgvdlgtenlyfq*sMAAPPQ
PVTHLIFDMDGLLLDTERLYSVVFQEICN
RYDKKYSWDVKSLVMGKKALEAAQIIIDV
LQLPMSKEELVEESQTKLKEVFPTAALMP
GAEKLIIHLRKHGIPFALATSSRSASFDM
KTSRHKEFFSLFSHIVLGDDPEVQHGKPD
PDIFLACAKRFSPPPAMEKCLVFEDAPNG
VEAALAAGMQVVMVPDGNLSRDLTTKATL
VLNSLQDFQPELFGLPSYE
```

* TEV cleave site

Growth medium, induction protocol: 10 µl of a glycerol stock was inoculated into 140ml of TB medium (supplemented with kanamycin 50µg/ml and chloramphenical 34µg/ml) in a 500 ml culture flask and incubated at 37°C o/n with shaking (275 rpm). The next day 10ml of overnight culture was added into 1L LB with 50 µg/ml of Kanamycin and 34 µg/ml of chloramphenicol (total 6L). The cells were cultured at 37°C until the OD_{600} reached 0.829 and then decreased the temperature to 18°C. IPTG was added at 0.1mM (final concentration) and kept the culture at 18°C for overnight.

Extraction buffer, extraction method: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 30 mM Imidazole. Complete Protease Inhibitor Cocktail Tablets (Roche) were added (one tablet/50ml buffer). The cells were harvested by centrifugation at 4,000 g for 10 min. The pellet from 1 L culture was resuspended in 25 ml of extraction buffer. The sample was homogenized by using the EmulsiFlex-05 homogenizer (Glen Creston) and then centrifuged at 37505 g. The supernatant was kept for further purification.

Column 1: Ni-NTA

Buffers:
Binding buffer: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 30 mM Imidazole
Washing Buffer: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 30 mM Imidazole
Elution Buffer I: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 60 mM Imidazole
Elution Buffer II: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 125 mM Imidazole
Elution Buffer III: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 250 mM Imidazole

Procedure: The column was packed by 6 ml of Ni-NTA slurry and equilibrated with 15 ml of binding buffer. The supernatant was loaded onto the column and the flow through was collected. The column was washed with 2x20 ml of of washing buffer. The protein was eluted with 10 ml of elution buffer I, II & III respectively.

Column 2: Superdex 200 Hiload 16/60

Buffers: 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 0.5 mM TCEP.

Procedure: Sample from Ni-NTA purification (wash II and elute I and II) was concentrated to 3 ml before loaded onto the AKTA Purifier at 4°C. Fractions were analyzed by SDS-PAGE and the most purified fractions were collected.

Concentration: 68.5 mg/ml

Enzymatic treatment: none

Mass spectrometry characterization: corresponds to theoretical mass, as determined by ESI-TOF MS.

Crystallisation: Crystals of HDHD1A were grown by vapor diffusion at 4°C, combined with microseeding procedures. Sitting drops consisting of 100 nl protein (68.5 mg/ml) and 100 nl crystallization solution (0.056 M sodium di-hydrogen phosphate and 1.334 M di-potassium hydrogen phosphate) were equilibrated against reservoirs containing the crystallization solution. After 1hr, drops were seeded with 20nl of 10^{-2} (v/v) dilution of a microseed suspension from initial crystal clusters grown in the same crystallization solution. Seeds were crushed using the Hampton Seed Bead Kit, and diluted into 50ul of crystallization solution. The crystal was cryo-protected in 30% glycerol before flash-cooling in liquid nitrogen.

Data Collection: Resolution: 2.0 Å; **X-ray source:** Diamond light source IO2.