

**Entry Clone Source:** Origene

**Entry Clone Accession:** NM\_152444

**SGC Construct ID:** ZADH1A-c501

**GenBank GI number:** gi|22748929

**Vector:** pNIC-CTHF. Details [[PDF](#)] ; Sequence [[FASTA](#)] or [[GenBank](#)]

**Amplified DNA sequence:**

CTTAAGAAGGAGATATACTATGATGATTG  
TTCAAAGAGTGGTATTGAATTCTCGACCT  
GGAAAAAAATGGTAATCCAGTGGCAGAGAA  
TTTCCGAATGGAAGAAGTCTATTACCAAG  
ATAATATTAATGAAGGACAAGTACAAGTT  
AGAACTCTTATCTTCTGTGGATCCTTA  
CATGCGTTGAGAATGAATGAAGACACTG  
GCACTGATTATATAACACCTTGGCAGCTA  
TCTCAAGTCGTTGATGGTGGAGGTATTGG  
AATTATAGAAGAAAGCAAACACACAAATT  
TGACTAAAGGCGATTGTGACTTCTTTC  
TATTGGCCCTGGCAAACCAAGGTTATTCT  
GGATGGAAATAGCCTGAAAAGGTAGACC  
CACAACTTGTGGATGGACACCTTCATAT  
TTCTTGGAGCTATAGGTATGCCCTGGTT  
GACTTCCTTGATTGGGATACAGGAAAAAG  
GTCATATAACTGCTGGATCTAATAAGACA  
ATGGTTGTCAGTGGGCGCAGGTGCCTG  
TGGATCTGTTGGCTGGCAGATTGGCATT  
TCTTAGGTTGTTCCAGAGTGGTGGGAATT  
TGTGGAACACATGAGAAATGCATCCTCTT  
GACCTCAGAACTGGGCTTGATGCTGCAA  
TTAATTATAAAAAAGACAATGTGGCAGAA  
CAGCTCCGTGAATCATGCCAGCTGGAGT  
GGATGTTATTTGACAATGTTGGTGGTA  
ACATCAGTGATAACAGTGATAAGTCAGATG  
AATGAGAACAGCCACATCATCCTGTGTGG  
TCAAATTCTCAGTACAACAAAGATGTGC  
CTTATCCTCCCCCGCTATCCCTGCTATA  
GAGGCAATCCAGAAAGAAAGAACATCAC  
AAGGGAAAGATTCTGGTATTAAATTATA  
AAGACAAATTGAGCCTGGCATTCTACAG  
CTGAGTCAGTGGTTAAAGAAGGAAAGCT  
AAAGATTAAGAGAGACGGTAATAATGGGT  
TGGAAAACATGGGAGCTGCATTCCAGTCC  
ATGATGACAGGAGGTAACATTGGAAAGCA  
GATAGTTGCATTCAGAAGAAATCGCAG  
AGAACCTCTACTTCCAATCGCACCACAT  
CACCACCATGATTACAAGGATGACGACGA  
TAAGTGAGGATCC

**Tags and additions:** C-terminal His-tag with TEV protease cleavage site

**Expressed protein sequence :**

MMIVQRVVLNSRPGKNGNPVAENFRMEEV  
YLPDNINEGQVQVRTLYLSVDPYMRCRMN  
EDTGTDYITPWQLSQVVDGGGIGIIEESK  
HTNLTKGDFVTSFYWPWQTKVILDGNSLE  
KVDPQLVDGHLSYFLGAIGMPGLTSLIGI

QEKGHITAGSNKTMVVSGAAGACGSVAGQ  
IGHFLGCSRGGICGTHEKCILLTSELGF  
DAAINYKKDNVAEQLRESCPAGVDVYFDN  
VGGNISDTVISQMNENSHIILCGQISQYN  
KDPVYPPPLSPAIEAIQKERNITRERFLV  
LNYKDKFEPGILQLSQWFKEGKLKIKETV  
INGLENMGAAFQSMMTGGNIGKQIVCISE  
EIAENLYFQshhhhhhdykddddk

**Host:** *E. coli* strain: BL21(DE3)-R3 pRARE2

**Growth medium, induction protocol:** 10µl of a glycerol stock was inoculated into 5ml of TB medium (supplemented with 50µg/ml Kanamycin, 34µg/ml Chloramphenicol) and cultured at 37°C overnight in a shaking incubator (275 rpm). Next day 0.75 ml of overnight culture was used to inoculate 1 litre of TB medium (6x) and grown at 37°C with vigorous shaking (160 rpm) until the culture reaches an OD<sub>600</sub> of 1.4. Temperature was reduced to 18°C, and cells were induced with IPTG at a concentration of 0.5 mM, and further cultivated for 16 hrs. Cells were harvested by centrifugation at 6500 rpm for 10 min, and the cell pellet was stored at -20°C until further use.

**Extraction buffer, extraction method:** **Lysis buffer:** 500 mM NaCl, 5% Glycerol, 50 mM HEPES pH 7.5, 5 mM Imidazole, Complete® protease inhibitors (Roche, 1tbl/50 ml). Frozen cell pellets were thawed and resuspended in a total volume of 30-40 ml of lysis buffer, and disrupted by using Avestin C-5 microfluidizer, and a supernatant containing the target protein was obtained by centrifugation at 21,000 (rpm) for 45 minutes.

**Column 1:** Ni-Sepharose 6 Fast Flow

**Buffers:** **Lysis buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 5 mM Imidazole; **Wash buffer:** 50 mM Tris-HCl pH 7.5, 500 mM NaCl, 5% Glycerol, 30 mM Imidazole; **Elution buffer:** 50 mM HEPES pH 7.5, 500 mM NaCl, 5% Glycerol, 250 mM Imidazole. **Note:** All the buffers contain 0.5mM TCEP.

**Procedure:** The column was packed with 2 ml of Ni-Sepharose 6 Fast Flow slurry and equilibrated with 15 ml of binding buffer. The supernatant was loaded onto the column and the column was washed with 20 ml of binding buffer and then 20 ml of washing buffer. The protein was eluted with 10 ml of elution buffer.

**Column 2:** HP Q column (ion exchange).

**PI value of protein:** 5.38

**Buffers:** **Buffer A:** 20 mM Tris-Cl pH 8.5, 50mM NaCl; **Buffer B:** 20 mM Tris-Cl pH 8.5, 2 M NaCl.

**Procedure:** The ZADH1A protein was applied to 5ml HP Q column in buffer A and eluted from the column by a linear gradient with buffer B.

**Enzymatic treatment:** TEV cleaved.

**Column 3:** Ni-NTA (TEV clean up)

**Buffer:** 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP

**Procedure:** Total 5 mgs of protein was cleaved with 300 µg of TEV protease at 4°C for 48 hours.

**TEV clean up:** The TEV cleaved protein was applied to a 1 ml Ni-NTA column, already equilibrated with gel filtration buffer (10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP). The flow through from the column was collected. The eluate from the column was monitored by SDS gel analysis.

**Concentration:** The ZADH1A protein (in buffer; 10 mM HEPES, pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP) was concentrated to 5 mg/ml using Vivaspin 10K concentrators and stored at -80°C.

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

**Crystallisation:** Crystals were grown by vapour diffusion in sitting drops at 20°C. Before crystallization setup protein was incubated with 5mM of NADP and 2.5mM of phenylbutazone. A sitting drop consisting of 50 nl protein and 100 nl well solution was equilibrated against well solution containing 2.1M DL-Malic acid pH 7.0. Crystals were mounted directly from the drop and flash-cooled in liquid nitrogen.

**Data Collection, Resolution:** 1.7 Å, **X-ray source:** Synchrotron SLS-X10SA, single wavelength.