

# HDAC7

PDB:2PQP

## Revision

**Revision Type:**created

**Revised by:**created

**Revision Date:**created

**Entry Clone Accession:**

**Entry Clone Source:**MGC

**SGC Clone Accession:**

**Tag:**N-terminal his tag with integrated thrombin protease site MGSSHHHHHHSSGLVPRGS

**Host:**E. coli BL21 (DE3) Codon Plus RIL (Stratagen)

## Construct

**Prelude:**

**Sequence:**

gsRAQSSPAAPASLSAPEPASQARVLSSSETPARTLPFTTGLIYDSVMLKHQCSCGDNSRHPEHAGRIQSIWSRLQERGLRSQCECL  
RGRKASLEELQSVHSERHVLLYGTNPLSRLKLDNGKLAGLLAQRMFVMLPCGGVGVDTDTIWNE LHSSNAARWAAGSVTDLAFKVAS  
RELKNGFAVVRPPGHHADHSTAMGFCFFNSVAIACRQLQQQSKASKILIVDWDVHHGNGTQQTFYQDPSVLYISLHRHDDGNFFPGS  
GAVDEVGAGSGEGFNVNVAWAGGLDPPMGDPEYLAAFRIVVMPIAREFSPDLVLVSAGFDAAEGHPAPLGGYHVSACFGYMTQQLM  
NLAGGAVVLALEGGHDLTAICDASEACVAALLGNRVDP LSEEGWKQKPNLNAIRSLEAVIRVHSKYWGCMQRLAS

**Vector:**p28a-LIC

## Growth

**Medium:**

**Antibiotics:**

**Procedure:**HDAC7 was expressed in E. coli BL21 (DE3) Codon Plus RIL in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin. Cells were grown at 37 oC to an OD600 of about 2 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG, final concentration 1 mM), and incubated overnight at 12 oC.

## Purification

### Procedure

The crude extract was cleared by centrifugation. 5 mM imidazole was added to the lysate. The sample was loaded onto 5 ml HiTrap Chelating column (Amersham Biosciences), charged with Ni<sup>2+</sup>. The column was washed with 10 CV of 20 mM Tris-HCl buffer, pH 8.0, containing 250 mM NaCl, 0.1 % CHAPS and 20 mM imidazole, and the protein was eluted with elution buffer (20 mM Tris-HCl, pH 8.0, 250 mM NaCl, 250 mM imidazole). Thrombin (Sigma) was added to combined fractions containing HDAC7 and incubated overnight at 4°C while dialyzing against 20 mM Tris-HCl, pH 8.0, 200 mM NaCl, and 5 mM β-mercaptoethanol. The protein was further purified to homogeneity by anion-exchange chromatography on Source 30Q column (10?10) (Amersham Biosciences), equilibrated with 20 mM Tris-HCl pH 8.0 and eluted with a linear gradient of NaCl up to 400 mM (15CV). Purification yield is about 0.75 mg of protein per 1L of culture.

## Extraction

### Procedure

Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80 °C. For the purification the cell paste was thawed and re-suspended in lysis buffer (1× PBS, 0.5 M NaCl, 5% glycerol, 0.1 % CHAPS, and 2.5mM β-mercaptoethanol) with protease inhibitor (0.1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 18,000 psi.

**Concentration:** 12 mg/mL

### Ligand

**MassSpec:** expected mass = 45465.4 Da, measured mass = 45465.7 Da

**Crystallization:** 2 mM NK146-I-306 (a.k.a. [LAQ 824](#), C<sub>22</sub>H<sub>25</sub>N<sub>3</sub>O<sub>3</sub>, dissolved in DMSO, compound kindly provided from N.P. Kwiatkowski and R. Mazitschek from the [Broad Institute](#)) was directly added to concentrated protein (12 mg/mL) and incubated for 30 min on ice prior to crystallization. HDAC7 was crystallized using the hanging drop vapor diffusion method at 20 °C by mixing 2 μl of the protein solution with 2 microL of the reservoir solution containing 13 % PEG 3350, 0.1 M Hepes pH 7.5, 10 % isopropanol, and 1 mM DTT.

### NMR Spectroscopy:

#### Data Collection:

#### Data Processing: