SIRT6

PDB:3K35

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:13477138
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPR*GS
Host:BL21(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

VNYAAGLSPYADKGKCGLPEIFDPPEELERKVWELARLVWQSSSVVFHTGAGISTASGIPDFRGPHGVWTMEERGLAPKFDTTFESA
RPTQTHMALVQLERVGLLRFLVSQNVDGLHVRSGFPRDKLAELHGNMFVEECAKCKTQYVRDTVVGTMGLKATGRLCTVAKARGLRA
CRGELRDTILDWEDSLPDRDLALADEASRNADLSITLGTSLQIRPSGNLPLATKRRGGRLVIVNLQPTKHDRHADLRIHGYVDEVMT
RLMKHLGLEIPAWDGPRVLERALPPLPRPPTPKLEPKEESPTRINGSIPAGPKQE

Vector:pET28a-LIC

Growth

Medium:
Antibiotics:
Procedure:SIRT6 was expressed in BL21(DE3)-R3-pRARE2 cells in Terrific Broth (TB) in the presence of 50 µg/mL of kanamycin at 37°C to an OD600 of 0.8. Cells were then induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 0.5 mM, and incubated overnight at 15°C.

Purification

Procedure
The clarified lysate was loaded onto 5 mL HiTrap Chelating column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM Hepes, pH 7.0, containing 250 mM NaCl and 5% glycerol, and the protein was eluted with elution buffer (20 mM Hepes buffer, pH 7.0, 250 mM NaCl, 5% glycerol and 250 mM imidazole). The protein was loaded onto Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 20 mM Pipes buffer, pH 6.5, and 150 mM NaCl, at flow rate 4 mL/min. Thrombin was added to combined fractions containing SIRT6 at 4°C overnight. The protein was further purified to homogeneity by ion-

exchange chromatography on Source 30S column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM Mes buffer, pH 6.5, and eluted with linear gradient of NaCl up to 500 mM concentration (30CV). Purification yield was 3 mg of the protein per 1L of culture.

Extraction

Procedure
Cells were harvested by centrifugation. The cell pellets were frozen in liquid nitrogen and stored at -80°C. The cell paste was thawed and resuspended in lysis buffer (1X PBS, pH 7.5, 0.5 M NaCl, 5% glycerol) with protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF). Cell lysis was accomplished by sonication (Virtis408912, Virsonic) on ice: the sonication protocol was 10 sec pulse at 80% maximal frequency (8.0), 10 second rest, for 5 minutes total sonication time. The cell lysate was clarified by centrifugation using a Beckman JLA-16.250 rotor at 15,500 rpms for 45. minutes at 4°C
Concentration:19 mg/mL
Ligand
MassSpec:expected MW is 35095.2 Da, measured MW is 35095.89 Da
Crystallization:10mg/mL purified SIRT6 was mixed with 10mM NAD(Sigma) and 3mM H3K9Ac(Tufts peptide synthesis) and 0.01mg/mL substilisin(Sigma) in 20mM Mes buffer, pH 6.5. SIRT6+APR was crystallized using hanging drop vapor diffusion method at 20°C by mixing 2 µl of the protein mix with 2 µl of the reservoir solution containing 1.8-1.9 M (NH4)2SO4, 2% PEG 400, Bis-Tris pH 5.6-6.2.
NMR Spectroscopy:
Data Collection:
Data Processing: