SIRT5

PDB:2B4Y

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|6912664
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPR*GS
Host:E.coli BL21 (DE3) codon plus RIL (Stratagen).

Construct

Prelude:
Sequence:

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure:SIRT5 was expressed in E.coli BL21 (DE3) codon plus RIL in TB medium in the presence of 50 µg/mL of kanamycin. Cell were grown at 37oC to an OD600 of 0.8 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM and incubated overnight at 15oC.

Purification

Procedure

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 resuspended in lysis buffer (1xPBS, pH 7.4, 0.25 M NaCl, 5 mM imidazol, 5% glycerol, 0.1% IPTG) with protease inhibitor (0.1mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.

Concentration:
Ligand
MassSpec:
Crystallization: Purified SIRT5 was complexed with NAD (Sigma) at 1:20 molar ratio of protein: NAD and crystallized using hanging drop vapor diffusion method drop at 20°C by mixing 1.5 µl of the protein solution with 1.5 µl of the reservoir solution containing 18% PEG 4000, 0.1M Hepes pH 7.5, 10% Isopropanol.
NMR Spectroscopy:
Data Collection:
Data Processing: