

GNPNAT1

PDB:2HUZ

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:GI:37620194

Entry Clone Source:MGC

SGC Clone Accession:

Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQ*G

Host:BL21 (DE3) codon plus RIL (Stratagene)

Construct

Prelude:

Sequence:

gMKPDETPMFDPSLLKEVDWSQNTATFSPAISPTHPGEGVLRLPLCTADLNRGFFKVLGQLTETGVVSPEQFMKSFEHMKKSGDYV
TVVEDVTLGQIVATATLIIHKFIHSCAKRGRVEDVVVSDCRGKQLGKLLSTLTLLSKKLNCYKITLECLPQNVGFYKKFGYTVS
EENYMCRRFLK

Vector:p28-MHL

Growth

Medium:

Antibiotics:

Procedure:GNPNAT1 was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin. Cell were grown at 37°C to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 15°C.

Purification

Procedure

The crude extract was cleared by centrifugation. 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 7.4, containing 500 mM NaCl and 50 mM imidazole, 5% glycerol, and the protein was eluted with elution buffer (20 mM HEPES, pH 7.4, 500 mM NaCl, 250 mM imidazole, 5% glycerol). The protein was dialyzed against 20 mM HEPES, pH 7.4, 500 mM NaCl, 5% glycerol in the presence of TEV protease. The dialyzed protein was passed through a 5 ml Ni HiTrap column and loaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 20 mM PIPES buffer, pH 6.5, and 250 mM NaCl, at flow rate 4 ml/min. The

pooled fractions containing GNP NAT1 was further purified to homogeneity by ion-exchange chromatography on Source 30S column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM PIPES pH 6.5, and eluted with linear gradient of NaCl up to 0.5 M concentration (20CV). Purification yield was 25 mg of the 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 resuspended in lysis buffer (50 mM HEPES, pH 7.4, 0.5 M NaCl, 5 mM imidazol, 2 mM β -mercaptoethanol, 5% glycerol) with protease inhibitor (0.1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.

Concentration: 59 mg/ml

Ligand

MassSpec: Expected mass = 20806.1 Da, measured mass = 20831.1 Da

Crystallization: Purified GNP NAT1 was crystallized using the sitting drop vapor diffusion method by mixing 1 μ l of protein solution with 1 μ l of the reservoir solution containing 25% PEG4000, 0.2 M ammonium sulfate, 0.1 M Na Acetate, pH 4.6, 0.1 M Yttrium chloride.

NMR Spectroscopy:

Data Collection:

Data Processing: