NAT13

PDB:2OB0

Revision

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Entry Clone Accession:GI:13376735
Entry Clone Source:MGC
SGC Clone Accession:MAK3_01:D6-APC008
Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPRGS
Host:E.coli BL21 (DE3) codon plus RIL (Stratagene)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsKGSRIELGDVTPHNIKQLKRLNQVIFPVSYNDKFYKDVLEVGELAKLAYFNDIAVGAVCCRVDHSQNQ
KRLYIMTLGCLAPYRRLGIGTKMLNHVLNICEKDGTFDNIYLHVQISNESAIDFYRKFGFEIIETKKNYYKRIEPADAHVLQKNLKV
PSGQNADVQKTDN

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure:NAT13 (MAK3) was expressed in E.coli BL21 (DE3) codon plus RIL in 2L Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin. Cell were grown at 37oC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, incubated overnight at 15oC.

Purification

Procedure
he crude extract was cleared by centrifugation at ~75000 x g for 60 minutes. The clarified lysate was loaded onto a 5ml Chelating Sepharose column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM HEPES buffer, pH 7.4, containing 0.5 M NaCl , 50 mM imidazole, 5% glycerol and 0.1% CHAPS, and the protein was eluted with elution buffer (20 mM HEPES, pH 7.4, 0.5 M NaCl, 250 mM imidazole, 5% glycerol, 0.1 % CHAPS). The protein was loaded on a Source30S column (10x10) (Amersham Biosciences), equilibrated with 20 mM HEPES buffer, pH 7.4, and eluted by linear gradient of NaCl. Purification yield was 8.2 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. 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 ß-mercaptoethanol, 5% glycerol, 0.1% CHAPS) with protease inhibitor (0.1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through a Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:11.8 mg/ml
Ligand
MassSpec:
Crystallization: Purified NAT13 (MAK3) was complexed with acetylcoenzyme A (AcCoA, Sigma) at 1:10 molar ratio of protein:AcCoA and crystallized using the sitting drop vapor diffusion method at 20 °C by mixing 1µl of the protein solution with 1 µl of the reservoir solution containing 0.1M Sodium Acetate pH 5.0, 16% PEG3350, 0.1% Dioxane, 10mM DTT.
NMR Spectroscopy:
Data Collection:
Data Processing: