Tb11.01.2886

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Revision

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Entry Clone Accession: Tb11.01.2886
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SGC Clone Accession: Tb11.01.2886:V5-L147:Mac041-B06
Tag:N-terminal tag: mgsshhhhhhssgrenlyfqg
Host:BL21-(DE3)-V2R-pRARE2

Construct

Prelude:
Sequence:

VDLELRVLEESDLSSHLELLGHLTEAPPLSGVELANIADMRRRAGIVTKVFCHQPTGGIVGSASLMIQPKFTRGGRAVGHIEDVVVD
PSYRGAGLGKALIMDLCEISRSKGCYKVILDSSEKSLPFYEKLGFRAHERQMRLDL

Vector:pET15-MHL

Growth

Medium:TB
Antibiotics:
Procedure: TbGNPNAT1 was expressed in E. coli BL21-(DE3)-V2R-pRARE2 resistant strain in Terrific Broth (TB) in the presence of ampicillin/chloramphenicol (100 microgram/mL and 34 microgram/mL respectively). A single colony was inoculated into 100mL of LB with of ampicillin/chloramphenicol (100 microgram/mL and 34 microgram/mL respectively) in a 250 mL baffled flask and incubated with shaking at 250 rpm overnight at 37 °C. The culture was transferred into 1.0 L of TB with ampicillin/chloramphenicol (100 microgram/mL and 34 microgram/mL respectively) and 0.15 mL of antifoam (Sigma) in a 1 L bottle and cultured using the LEX system to an OD600 of 5-6, cooled to 15 °C, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 °C.

Purification

Procedure
The cleared lysate was loaded onto a 1.0-2.5 mL Ni-NTA (Qiagen) column (pre-equilibrated with Binding Buffer) at approximately 1.5-2.0 mL/min. The Ni-NTA column was then washed with 150 mL of Wash Buffer at 2-2.5 mL/min. After washing, the protein was eluted with Elution Buffer. The eluted sample was applied to a Sephadex S200 16/60 gel filtration column pre-

equilibrated with Gelfiltration Buffer. The fractions corresponding to the eluted protein peak were collected and further concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). The protein sample identity and purity were evalulated by mass spectroscopy and SDS-PAGE gel. The concentrated protein was stored at 4 degC. For long term storage, the protein was flash frozen and stored at -80 degC.

Extraction

Procedure
The culture was harvested by centrifugation. Pellets from 1 L of culture were resuspended to approximately 40 mL/L of cell culture in Binding Buffer with the addition of protease inhibitors (1 mM benzamidine and 1 mM phenylmethyl sulfonyl fluoride (PMSF)). Resuspended pellets stored at -80 degC were thawed overnight at 4 °C on the day before purification. Prior to sonication, each pellet from 1 L of culture was pretreated with 0.5 % CHAPS and 500 units of benzonase for 40 minutes at room temperature. After 6 minutes sonication, the cell lysate was centrifuged using a Beckman JA-25.25 rotor at 24,000 rpms for 20 minutes at 4 degC.
Concentration:
Ligand
MassSpec:
Crystallization:sitting drop vapor diffusion, Crystallization buffer: 20% PEG3350, 0.2 M di-Ammonium Tartrate, at 293K.
NMR Spectroscopy:
Data Collection:
Data Processing: