

INMT

PDB:2A14

Revision

Revision Type:created

Revised by:created

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Entry Clone Accession:GI:66933018

Entry Clone Source:MGC

SGC Clone Accession:

Tag:N-terminal His-tag with integrated thrombin protease site: mgsshhhhhssglvprgs

Host:E.coli BL21 (DE3) codon plus RIL (Stratagene)

Construct

Prelude:

Sequence:

gsMKGGFTGGDEYQKHFLPRDYLATYYSFDGSPSPEAEMLKFNLECLHKTFGPGGLQGDTLIDIGSGPTIYQVLAACDSFQDITLSD
FTDRNREELEKWLKKEPGAYDWTPAVKFACELEGNSGRWEEKKLRRAVKRVLKCDVHLGNPLAPAVLPLADCVLTLLAMECACCS
LDAYRAALCNLASLLKPGGHLVTTVTLRLP SYMVGKREFSCVALEKGEVEQAVLDAGFDIEQLLHSPQSYSVTNAANNGVCCIVARK
KPGP

Vector:p28a-LIC

Growth

Medium:

Antibiotics:

Procedure:INMT was expressed in E.coli BL21 (DE3) codon plus RIL in Terrific Broth (TB) in the presence of 50 µg/mL of kanamycin at 37°C to an OD600 of 1.5. Cells were then 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 Tris-HCl buffer, pH 8.0, containing 250 mM NaCl and 50 mM imidazole, and the protein was eluted with elution buffer (20 mM Tris-HCl, pH 8.0, 250 mM NaCl, 250 mM imidazole). The protein was loaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 20 mM Tris-HCl buffer, pH 8.0, and 150 mM NaCl, at flow rate 4 mL/min. Thrombin (Sigma) was added to combined fractions containing INMT and incubated overnight at

40C. The protein was further purified to homogeneity by ion-exchange chromatography on Source 30Q column (10x10) (Amersham Biosciences), equilibrated with buffer 20 mM Tris-HCl, pH 8.0, and eluted with linear gradient of NaCl up to 500 mM concentration (20CV). Purification yield was 6.7 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 (1 XPBS, 0.25 M NaCl, 5 mM imidazol, 2 mM β -mercaptoethanol, 5% glycerol) 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 INMT was complexed with S-adenosyl-L-homocysteine (SAH) (Sigma) at 1:5 molar ratio of protein: SAH and crystallized using the sitting drop vapor diffusion method at 20 °C by mixing 1 μ l of the protein solution with 1 μ l of the reservoir solution containing 2.0 M ammonium sulfate, 0.2 M K/Na tartrate, 0.1 M Sodium Citrate, pH 5.6.

NMR Spectroscopy:

Data Collection:

Data Processing: