

# INMT

**PDB:**2A14

## Revision

**Revision Type:**created

**Revised by:**created

**Revision Date:**created

**Entry Clone Accession:**GI:66933018

**Entry Clone Source:**MGC

**SGC Clone Accession:**

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

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

## Construct

**Prelude:**

**Sequence:**

gsMKGGFTGGDEYQKHFLPRDYLATYYFDGSPSPEAEMLKFNLÉCLHKTFGPGLQGDTLIDIGSGPTIYQVLAACDSFQDITLSD  
FTDRNREELEKWLKKEPGAYDWTPAVKFACELEGNSGRWEEKELRAAVKRVLKCDVHLGNPLAPAVLPLADCVLTLLAMECACCS  
LDAYRAALCNLASLLKPGGHLVTTVTLRLPSYMVKGREFSCVALEKGEVEQAVLDAGFDIEQLLHSPQSYVTNAANNGVCCIVARK  
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

4oC. 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  $\beta$ -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  $\mu$ l of the protein solution with 1  $\mu$ 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: