

# TPMT

**PDB:**2BZG

## Revision

**Revision Type:**created

**Revised by:**created

**Revision Date:**created

**Entry Clone Accession:**62953142

**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 (Stratagen).

## Construct

**Prelude:**

**Sequence:**

**Vector:**p28a-LIC

## Growth

**Medium:**

**Antibiotics:**

**Procedure:**TPMT was expressed in E.coli BL21 (DE3) codon plus RIL in M9 minimal medium in the presence of 50 µg/mL of kanamycin. Cell were grown at 37oC to an OD600 of 0.8 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, in the presence of 50 mg/L of SeMet and incubated overnight at 15oC.

## Purification

### Procedure

## 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 (1X phosphate-buffered-saline, 0.25 M NaCl, 5 mM imidazol, 2 mM  $\beta$ -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:**

**Ligand**

**MassSpec:**

**Crystallization:** Purified TPMT was crystallized in the presence of S-adenosyl-L-homocysteine (SAH) using the hanging drop vapor diffusion method at 20 °C by mixing 1.5 µl of the protein solution with 1.5 µl of the reservoir solution containing 24% PEG3350, 0.2 M KCNS, 0.1 M BisTris propane, pH 7.0.

**NMR Spectroscopy:**

**Data Collection:**

**Data Processing:**