

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: MGSSHHHHHHSSGLVPRGS

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 37°C 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 15°C.

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 β-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: