= PDB Code =
3S4Y

= Ligands =
THIAMINE DIPHOSPHATE

= Entry clone accession =
BC068460

= Entry clone source =
MGC AU57-A12

= SGC clone accession =
HPC102-A10

= Tag =
N-terminal His6-tag

= Construct comments =
TPK1:G15-S243

= Construct sequence =
MHHHHHHSSGRENLYFQGGNLKYCLVILNQPLDNYFRHLWNKALLRACADGGANRLY
DITEGERESFLPEFINGDFDSIRPEVREYYATKGCELISTPDQDHTDFTKCLKMLQKKIEEK
DLKVDVIVTLGGLAGRFDQIMASVNTLFQATHITPFPIIIIQEESLIYLLQPGKHRLHVDTG
MEGDWCGLIPVGQPCMQVTTTGLKWNLTNDVLAFGTLVSTSNTYDGSGVVTVETDHPL
LWTMAIKS

= Vector =
pET28-MHL

= Expression host =
BL21-V2R-pRARE2

= Growth method =
LEX Bubbling. The target protein was expressed in E. coli by inoculating each 50 mL of overnight culture grown in Luria-Bertani medium into a 1.8 L of Terrific Broth medium in the presence of 50 mg/L kanamycin and 30 mg/L chloramphenicol at 37 degree. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 degree and the culutre was induced with 1 mM IPTG. The cells were allowed to grow overnight before harvested and flash frozen in liquid nitrogen and stored at -80 degree.

= Extraction buffers =
50mM Tris/HCl pH7.8; 500mM NaCl; 5% Glycerol; 5mM BME (freshly added 1mM PMSF; 10U/ml Benzonase; 0.5% CHAPS)

= Extraction procedure =
Frozen cells from 4L TB culture were thawed and resuspended in 5mL extraction buffer per gram of cell pellets with freshly added 0.5% CHAPS, and supplemented with protease inhibitor 1mM PMSF, and 10U/mL benzonase (Sigma Catalog # E1014, 250U/uL), and lysed using microfluidizer, 16000 PSI, once.

= Purification buffers =
Washing buffer: 50 mM Tris/HCl 7.8; 500mM NaCl; 5% Glycerol; 5mM BME; 25 mM or 50 mM Imidazole
Elution buffer: 50 mM Tris/HCl 7.8; 500mM NaCl; 5% Glycerol; 5mM BME; 300 mM Imidazole
Gel Filtration Buffer: 20mM HEPES, 7.30, 300mM NaCl, 1mM TCEP

= Purification procedure =
The lysate was centrifuged at 15,000 rpm for 45 minutes and the supernatants were mixed with 4 mL 50% flurry of Ni-NTA beads and incubated at 4 degree on rotary shaker for one hour. The mixture was then centrifuged at 2300 rpm for 5 min and the supernant discarded. The beads were then washed with 50 mL binding buffer containing 25 mM and 50 mM Imidazole. Bound proteins were eluted using 15 mL elution buffer. The protein is further purified by a Superdex-75 gel filtraton column pre-equilibrated with gel filtration buffer. Fractions containing the target protein were pooled and concentrated using Amicon Ultra-15 centrifugal filter (mwco 10 kDa). The purity of the preparation is tested by SDS-PAGE to be greater than 90%.

= Protein stock concentration =
27.4 mg/mL

= Mass spec =
The protein expected 27890.8, measured 27891.1

= Crystallization =
Crystal used for structure refinement was grown in SGC-I screen condition C08, i.e. in 25% PEG 3350, 0.2M NH4SO4, 0.1M NaCaco pH 5.5, in sitting drop setup, using 0.5uL protein + 0.5uL well solution against 100 uL reservoir buffer at room temperature.

Crystals grow to a mountable size within two days.
No cryo used for mounting these crystals