

MCTS1

PDB:3R90

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:Q9ULC4 / BC001013

Entry Clone Source:MGC AU9-E2

SGC Clone Accession:HPC0AA-D07

Tag:C-terminal his tag (AHHHHHHH)

Host:BL21(DE3)-V2R-pRARE2

Construct

Prelude:

Sequence:

MFKKFDEKENVSNCIQLKTSVIKGIKNQLIEQFPGIEPWLNQIMPKKDPVKIVRCHEHIEILTVNGELLFFRQREGPFYPTLRLLHK
YPFILPHQQVDKGAIKFVLSGANIMCPGLTSPGAKLYPAAVDITIVAIMAAGAAHALCVGVMKMSAEDIEKVNKGIGIENIHYLNDGL
WHMKTYKahhhhhh Underlined letters : susbstiuted residues E137A / K139A / Q140A

Vector:pNIC-CH

Growth

Medium:Terrific Broth

Antibiotics:

Procedure:LEX Bubbling. The target protein was expressed in E. coli by inoculating 40~50 mL of overnight culture grown in Luria-Bertani medium into a 2 L of Terrific Broth medium in the presence of 50 µg/mL kanamycin and 30 µg/mL chloramphenicol at 37 °C. When OD600 reached ~3.0, the temperature of the medium was lowered to 17 °C and the culture was induced with 0.5 mM IPTG. The cells were allowed to grow overnight before harvested and flash frozen in liquid nitrogen and stored at -80 °C.

Purification

Procedure

The cell lysate was then centrifuged to remove insoluble material, and the supernatant was loaded onto a DEAE-cellulose (DE52, Whatman, MA, USA) anion-exchange resin followed by a nickel-NTA agarose column (Qiagen, MD, USA). Bound proteins were eluted with a elution. The eluted sample was then dialyzed and subjected to cation-exchange chromatography using a HiTrap SP column (GE Healthcare, NJ, USA) previously equilibrated with the same buffer used for dialysis. Protein was eluted with a linear gradient of 0-500 mM NaCl, and further purified by

size exclusion chromatography on a Superdex 75 16/60 column (GE Healthcare, NJ, USA). The peak fractions containing MCTS-1 protein were pooled, concentrated using amicon centrifugal filter and stored at -80 °C before crystallization.

Extraction

Procedure

Frozen cells were thawed and suspended in 150 mL the binding buffer and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 2 μ L (Sigma Catalog # E1014, 250U/ μ L) benzonase, and lysed using a Microfluidizer at 16,000 psi.

Concentration: 30 mg/mL

Ligand

MassSpec:

Crystallization: Native crystals : 2.5M AmSO₄; Bis-Tris propane pH 7.0

SeMet-derivatized crystals : 2.5M AmSO₄; Bis-Tris propane pH 7.0; 20% PEG3350

NMR Spectroscopy:

Data Collection:

Data Processing: