

Molecular Biology

Entry Clone Accession: IMAGE 3493526

Entry Clone Source: IMAGE

SGC Construct ID: MMADHCMMA-c001

Protein Region: P129-N296

Vector: pNIC28-Bsa4

Tag: N-6HIS;N-TEV

Host: BL21(DE3)-R3

Sequence (with tag(s)):

MHHHHHHSSGVDLG TENLYFQSMPVEQEINSAETYFESAKVECAIQTCPELLRRDFESLF
PEVANSKLMILTVTQKTENDMTVWSEEVEVEREVLLEKFISGAKEICYALRAEGYWADFI
DPSSGVAFFGPYTNNTLFETDERYRHLGFSVDDLGCCKVIRHSLWGTHVVVGSIFTNATA
DSSIMRKLSGN

Sequence after tag cleavage:

SMPVEQEINSAETYFESAKVECAIQTCPELLRRDFESLFPEVANSKLMILTVTQKTENDMT
VWSEEVEVEREVLLEKFISGAKEICYALRAEGYWADFIDPSSGVAFFGPYTNNTLFETDER
YRHLGFSVDDLGCCKVIRHSLWGTHVVVGSIFTNATADSSIMRKLSGN

DNA Sequence:

CATATGCACCATCATCATCATCATTCTTCTGGTGTAGATCTGGGTACCGAGAACCTGTA
CTTCCAATCCATGCCTGTTGAACAAGAAATTAACAGTGCAGAACTTACTTTGAAAGT
GCCAAAGTTGAGTGTGCGATCCAGACATGTCCAGAGCTGCTGCGGAGAGATTTTGAG
TCACTTTTTCCAGAAGTGGCTAATAGCAAATAATGATCCTGACTGTAACCCAGAAAA
CTGAAAATGACATGACTGTCTGGAGTGAAGAGGTGGAGGTTGAGAGAGAGAGGTGCTCT
TGGAAAAGTTTCATCAGTGGTGCTAAGGAGATCTGCTATGCCCTTCGGGCTGAAGGATA
TTGGGCTGACTTCATTGACCCATCATCTGGTGTGGCATTTTTTTGGACCATATACCAACA
ACACACTTTTTTGAAACAGATGAACGGTACCGGCATTTAGGATTCTCAGTTGACGACCT
TGGCTGCTGTAAAGTGATCCGTCACAGTCTCTGGGGTACTCATGTGGTTGTAGGAAGT
ATCTTCACTAACGCAACAGCAGACAGCAGTATCATGAGGAAGCTCAGTGGAACTAG
CAGTAAAGGTGGATACGGATCCGAA

Protein Expression

Medium: TB

Antibiotics: Kanamycin

Procedure: Protein was expressed in BL21(DE3)R3-Rosetta *E. coli* cells grown in TB medium with 50 µg/ml kanamycin, with induction of IPTG overnight at 18°C. Cell pellets were harvested, sonicated in lysis buffer (50 mM HEPES pH7.5, 10 mM imidazole, 500 mM NaCl, 5% glycerol) and insoluble material removed by centrifugation.

Protein Purification

Procedure: The supernatant was purified by affinity (Ni-Sepharose; GE Healthcare) and size-exclusion (Superdex 200; GE Healthcare) chromatography.

Concentration: 23.0 mg/ml

Mass-spec Verification: Yes

Structure Determination

Crystallization: SeMet crystals of mouse MMADHC were grown by vapor diffusion at 4°C in sitting drops containing 100 nl of protein (23 mg/ml) and 50 nl mother liquor (28% PEG3350, 100 mM bis-tris pH 6.5, 250 mM NaCl). Native crystals were grown by vapor diffusion at 4°C in sitting drops containing 75 nl of protein (23 mg/ml) and 75 nl mother liquor (22% polyacrylic acid 5100, 100 mM HEPES pH 7.5, 20 mM MgCl₂). Crystals were cryo-protected in mother liquor containing ethylene glycol (25% v/v) and flash-cooled in liquid nitrogen.

Protein Concentration: 23.0mg/ml

Data Collection: *Beamline:* Dmnd I04; *Resolution:* 2.3 Å; Diffraction data were collected at the Diamond Light Source beamlines I04, and processed using xia2.