

Structure MBD2

PDB Code 6C1A, 6C1T, 6C1U, 6C1V, 6CNP, 6CNQ

Entry clone accession BC032638

Entry clone source AT55-D9

Tag N-terminal hexahistidine tag with integrated TEV protease cleavage site:
mhhhhhssgrenlyfqg.

Construct sequence Mhhhhhssgrenlyfqg
ATESGKRMDCPALPPGWKKEEVIRKSGLSAGKSDVYYFSPSGKKFRSKPQLARYLGNTV
DLSSFD FRTGKMMPSKLQK

Vector pET28-MHL

Expression host E. coli BL21(DE3)-V2R-pRARE2

Growth medium TB

Growth method A fresh transformation was used to inoculate 50 mL LB media containing 50 µg/mL kanamycin and 30 µg/mL chloramphenicol. The culture was grown overnight at 37°C with shaking. The next day this starter culture was used to inoculate 2L of TB growth medium. The culture was grown in LEX at 37°C to OD600 of 3.1. IPTG-based induction was carried out according to the manufacturer's protocol. The temperature was reduced to 16°C and the culture was incubated for a further 18 hours before harvesting the cells.

Extraction buffers Lysis buffer: 20mM Tris pH=8.0, 500mM NaCl, 5% Glycerol, 0.1% NP40, 1mM PMSF, 5 units/mL Benzonase nuclease, 0.2 mM β-mercaptoethanol.

Extraction procedure Cells were harvested by centrifugation and pellets were stored in -80°C. Prior to purification, the cell pellet was resuspended in lysis buffer. Cells were disrupted by sonication (10 minutes) and samples were centrifuged for 60 min at 70000 g.

Purification buffers NiNTA Elution buffer (EB): 20mM Tris-HCl pH 7.5, 500mM NaCl, 250 mM Imidazole, 1mM DTT

Anion exchange buffer: A: 20mM Tris pH 7.5, 20mM NaCl, 1mM DTT, B: 20mM Tris pH7.5, 1M NaCl, 1mM DTT.

Gel filtration buffer: 20mM Tris pH 7.5, 150 mM NaCl, 1mM DTT.

Purification procedure Column 1: Affinity purification, open Ni-NTA column Procedure: The supernatant was incubated with 4mL of 50% slurry Ni-NTA beads by rocking. After 1 hour incubation at 4°C, the beads were washed with 50 mL of lysis buffer. The protein was eluted using ~20mL EB. The Column 2: anion exchange, HiTrap SP FF 5ml. Fraction from Ni-NTA column was combined and diluted up to 4 folds of volume by anion exchange buffer A. All the samples were loaded to HiTrap SP FF column at 3ml/min. Then sample was eluted by gradient increasing the concentration of buffer B. Then the fractions containing protein were identified on a SDS-PAGE gel.

Column 3: Superdex 75.

Protein stock concentration 10mg/ml.

Crystallization 6C1A: 20% PEG3350, 0.2 M ammonium chloride

6C1T: 27% PEG3350, 0.1 M Bis-Tris, 0.15 M ammonium sulfate, pH 5.5

6C1U: 20% PEG3350, 0.2 M potassium dihydrogen phosphate

6C1V: 25% PEG3350, 0.1 M Bis-Tris, 0.1 M ammonium sulfate, pH 6.5

6CNP: 8% PEG550MME, 8% PEG20000, 0.2M calcium acetate

6CNQ: 20% PEG3350, 0.2 M ammonium formate

Data collection