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| Structure | MBD1 |
| PDB Code | 6D1T |
| Entry clone accession | AF078830 |
| Entry clone source | AU94-F10 |
| Tag | N-terminal hexahistidine tag with integrated TEV protease cleavage site: mhhhhhhssgrenlyfqg. mhhhhhhssgrenlyfqg |
| Construct sequence | MAEDWLDCPALGPGWKRREVFRKSGATCGRSDTYYQSPTGDRIRSKVELTRYLGPACDLTLFDFKQGII |
| 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 37ºC with shaking. The next day this starter culture was used to inoculate 2 L of TB growth medium. The culture was grown in LEX at 37 ºC to $OD_{600}$ of 1.0. IPTG-based induction was carried out according to the manufacturer's protocol. The temperature was reduced to 16 ºC and the culture was incubated for a further 18 hours before harvesting the cells. |
| Extraction buffers | **Lysis buffer**: 20 mM, Tris pH 7.5, 500 mM NaCl, 5% Glycerol, 0.1% NP40, 1 mM PMSF<br>Cells were harvested by centrifugation and pellets were stored in -80 ºC. |
| Extraction procedure | 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)**: 20 mM Tris-HCl pH 7.5, 500 mM NaCl, 250 mM Imidazole<br>**Gel Filtration buffer**: 20 mM Tris pH 7.5,150 mM NaCl, 1 mM DTT. |
| Purification procedure | Column 1: Affinity purification, open Ni-NTA column Procedure: The supernatant was incubated with 6mL of 50% slurry Ni-NTA beads by rocking. After 30 min incubation at 4ºC, the beads were washed with 50 mL of lysis buffer. The protein was eluted using ~20 mL EB. The Column 2: Gel Filtration (Superdex S75 16/60 Hi-Load, GE Healthcare). Then the fractions containing protein were identified on a SDS-PAGE gel. |
| Protein stock concentration | 10 mg/ml. |
| Crystallization | 30% PEG-550-MME, 0.1 M magnesium chloride, 0.1 M HEPES |
| Data collection | |