PHF13

PDB:3O7A

Revision

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Entry Clone Accession:BC038516
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SGC Clone Accession:PHF13_12; plate JMC026:G05
Tag:N-terminal tag: MGSSHHHHHHSSRENLYFQG
Host:BL21 (DE3) Codon plus RIL (Stratagene)

Construct

Prelude:
Sequence:

DDDSWDLVTCFCMKPFAGRPMIECNECHTWIHLSCAKIRKSNVPEVFVCQKCRDS

Vector:pET28-MHL

Growth

Medium:LB media containing 50 µg/mL kanamycin and 30 µg/mL chloramphenicol and 10µM $ZnCl_2$
Antibiotics:
Procedure:A fresh transformation was used to inoculate 20 mL LB media containing 50 µg/mL kanamycin and 30 µg/mL chloramphenicol and 10µM $ZnCl_2$. 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 containing 100µM $ZnCl_2$. The culture was grown in LEX at 37°C to OD_{600} of 2.3. The temperature was reduced to 14°C and IPTG-based induction (1mM) was carried out according to the manufacturer's protocol. The culture was incubated for a further 18 hours before harvesting the cells. Cells were harvested by centrifugation and pellets were stored at -80°C.

Purification

Procedure
Column 1: Affinity purification, open Ni-NTA column
Procedure: The supernatant was incubated with 6mL of 50% slurry Ni-NTA beads (buffer is changed to lysis buffer prior to use) by rocking. After 1 hour incubation at 4ºC, the bead mixture was transferred to an empty column and washed with 200 mL of lysis buffer. The protein was eluted using ~20mL EB.

Column 2: Size Exclusion, HiLoad 16/60 Superdex 75 Prep Grade
Procedure: The elution from the NiNTA column was concentrated using 15 mL concentrators with a 3K Da molecular weight cut-off (Amicon Ultra-15, Millipore). The concentrated protein was loaded onto the size exclusion column at a flow rate of 1 mL/min, and 2 ml fractions were collected. The fractions containing protein were identified on an SDS-PAGE gel. Pool the fractions together and concentrated it using the 15 mL concentrators with a 3K Da molecular weight cut-off (Amicon Ultra-15, Millipore).

Extraction

Procedure
Prior to purification, the cell pellet was resuspended in lysis buffer. Cells were disrupted by sonication (100 watts, 10 minutes total time using 10 second pulses followed by 10 second rest) on ice and samples were centrifuged for 60 min at 70 000xg.
Concentration: The final concentration was 10.9 mg/ml. The protein yield was approximately 11 mg per litre of bacterial culture.
Ligand
MassSpec:
Crystallization: Crystals of PHF13-H3K4me3 were grown at 291 K using the sitting drop method by mixing equal volumes of 0.1M Tris-HCl pH 7.5; 1.5M Na-Citrate, PEG400 6%, 5mM H3K4me3 peptide was present in the protein stock solution as binding partner. The crystals were cryoprotected by cryoprotectant consisting of 100% reservoir solution and 18% glycerol.
NMR Spectroscopy:
Data Collection:
Data Processing: