CHD1

PDB:4NW2

Revision

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Entry Clone Accession:NP_001261.2 GI:68299795
Entry Clone Source: Open Biosystems
SGC Clone Accession:JMC034-H11
Tag:MHHHHHHSSGRENLYFQG
Host:BL21(DE3)-V2R-pRARE2

Construct

Prelude: Tag not removed
Sequence:

MHHHHHHSSGRENLYFQGEEEFETIERFMDCRIGRKGATGATTTIYAVEADGDPNAGFEKNKEPGEIQYLIKWKGWSHIHNTWETEE
TLKQQNVRGMKKLDNYKKKDQETKRWLKNASPEDVEYYNCQQELTDDLHKQYQIVERIIAHSNQKSAAGYPDYYCKWQGLPYSECSW
EDGALISKKFQACIDEYFSR

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:LEX Bubbling. The target protein was expressed in E. coli by inoculating 100 mL of overnight culture grown in Luria-Bertani medium into a 2L of Terrific Broth medium in the presence of 50 mg/mL kanamycin and 30 mg/mL chloramphenicol at 37 degree. When OD600 reached ~2.0, the temperature of the medium was lowered to 16 degree and the culture was induced with 0.4 mM IPTG. The cells were allowed to grow overnight before harvested and flash frozen in liquid nitrogen and stored at -80 degree

Purification

Procedure
The lysate was centrifuged at 16,000 rpm for 45 minutes and the supernatants were mixed with 8 mL 50% flurry of Ni-NTA beads and incubated at 4 degree on rotary shaker for one hour. The mixture was then centrifuged at 2000 rpm for 5 min and the supernatant discarded. The beads were then washed with 50 mL washing buffer, and finally 25 mL the elution buffer. The protein further purified by a Superdex-75 gel filtration column. Fractions containing the protein were

collected and concentrated with Amicon Ultra-15 centrifugal filter. The purity of the preparation is tested by SDS-PAGE to be greater than 95%.

Extraction

Procedure
Frozen cells from 2L TB culture were thawed and resuspended in 200 mL extraction buffer and lysed using sonicator.
Concentration:50 mg/mL
Ligand
Influenza NS1 C-terminal tail trimethylated at K229PKQKRKMARTARSK(me3)V**MassSpec:**
Crystallization: Crystallization was setup in sitting drops with Red Wings screens initially. Diffracting crystals were from initial screen plate for Red Wings A11.Crystal used for structure determination was grown in 10% PEG 8000, 0.2M MgCl2, 0.1M Tris buffer at pH 8.5.Crystals grow to a mountable size within 1 week
NMR Spectroscopy:
Data Collection:
Data Processing: