BRD3: Human bromodomain containing protein 3 (domain 2)

PDB:2OO1

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:gi|11067749
Entry Clone Source: Origene
SGC Clone Accession:
Tag:mhhhhhhssgvdlgtenlyfq*s(m) TEV-cleavable (*) N-terminal his6 tag.
Host: Rosetta (DE3)

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmGKLSE HLRYCDSILREMLSKKHAAYAWPFYKPVD AEALELHDYHDIIKHPMDLSTVKRKMDG
R EYPDAQGFAADVRLMFSNCYKYNPPDHEV VAMARKLQDVFEMRFAKMP

Vector:pNIC28-Bsa4

Growth

Medium:
Antibiotics:
Procedure:3ml from a 50 ml overnight culture was used to inoculate each of two flasks containing 1 litre of LB media containing 50 µg/ml kanamycin and 34 µg/ml chloramphenicol. Cultures were grown at 37°C to an OD600 of ~0.4 and then cooled to 18°C. Expression was induced for 4 hours using 0.5mM IPTG at an OD600 of 0.8. The cells were collected by centrifugation and the pellet resuspended in binding buffer and frozen. Binding buffer: 50mM HEPES pH 7.5; 500 mM NaCl; 5% glycerol; 5 mM imidazole.

Purification

Procedure

Extraction

Procedure
Cell pellets in binding buffer plus 1 mM PMSF, 0.5 mM TCEP were lysed using a high pressure cell disrupter. The lysate was centrifuged at 17,000 rpm for 30 minutes and the supernatant collected for purification. Prior to purification the lysate was passed through a DE52 column (10g/L resin) to remove DNA.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained in sitting drops using the vapor diffusion method by mixing 150nl of the concentrated protein with 50nl of a well solution 0.2 M NaF; 0.1M BTProp pH 7.5; 20.0% PEG 3350; 10.0% EtGly. Crystals appeared after several days at 4°C.
NMR Spectroscopy:
Data Collection: Crystals were cryo-protected using the well solution and 25% ethylene glycol and flash frozen in liquid nitrogen. Diffraction data (1.6 Å) were collected at the Swiss light source (SLS) at a single wavelength (0.99 nm).
Data Processing: