

KDM2B

PDB:4O64

Revision

Revision Type:created

Revised by:created

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Entry Clone Accession:Q8NHM5

Entry Clone Source:MGC :CM31-H4

SGC Clone Accession:KDM2B_L1 (JMC077:F02): R607-N723

Tag:N-terminal tag: MHHHHHHSSGRENLYFQG

Host:E. coli BL21(DE3)-V2R-pRARE2

Construct

Prelude:

Sequence:

MHHHHHHSSGRENLYFQG RRRRTRCRKCEACLRTECGECHFCKDMKKFGGPGRMKQSCIMRQCIAPVLPHTAVCLVCGEAGKEDTV
EEEEGKFNLMLECSICNEIHPGCLKIKESGVVNDELPCWCEPKCN

Vector:pET28-MHL

Growth

Medium:

Antibiotics:

Procedure: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 1. IPTG-based induction was carried out according to the manufacturer's protocol. The temperature was reduced to 14°C and the culture was incubated for a further 18 hours before harvesting the cells.

Purification

Procedure

IMAC: Unclarified lysate was mixed with 2 mL of Ni-NTA superflow Resin (Qiagen) per 40 mL lysate. The mixture was incubated with mixing for at least 45 minutes at 4°C. The mixture was then loaded onto an empty column (BioRad) and washed with 50 mL wash buffer. Samples were eluted from the resin by exposure to 2-3 column volumes (approx. 10-15 mL) of elution buffer. Concentration of eluted protein was estimated by OD280.

Gel filtration chromatography: An XK 26x65 column (GE Healthcare) packed with HighLoad Superdex 75 resin (GE Healthcare) was pre-equilibrated with gel filtration buffer for 1.5 column volumes using an AKTA explorer (GE Healthcare) at a flow rate of 1.0 mL/min. The dialyzed sample from the IMAC step (approx. 15 mL) was loaded onto the column at 1.5 mL/min, and 2mL fractions were collected into 96-well plates (VWR 40002-012) using peak fractionation protocols. Fractions observed by a UV absorption chromatogram to contain the protein were pooled.

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.

Concentration: Purified proteins were concentrated using 15 mL concentrators with a 5,000 molecular weight cut-off (Amicon Ultra-15, UFC900524, Millipore) at 3750 rpm, typically resulting in a final concentration around 10mg/mL.

Ligand

MassSpec:

Crystallization: Recombinant human KDM2B CXXC and PHD domain was crystallized using the sitting drop vapour diffusion method at 18 °C. The crystals were obtained in a buffer containing 25% PEG-8000, 0.2 M sodium chloride, 0.1 M tris, pH 8.5. Crystals were soaked in a cryoprotectant consisting of 100% reservoir solution and 15% glycerol.

NMR Spectroscopy:

Data Collection:

Data Processing: