WDR5-Histone H3

PDB:2H9M

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:NP_060058
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal his tag with integrated thrombin protease site MGSSHHHHHHSSGLVPRGS
Host:E. coli BL21 (DE3) Codon Plus RIL (Stratagen)

Construct

Prelude:
Sequence:

gsMATEEKKPETEAARAQPTPSSSATQSKPTPVKPNYALKFTLAGHTKAVSSVKFSPNGEWLASSSADKLIKIWGAYDGKFEKTISG
HKLGISDVAWSSDSNLLVSASDDKTLKIWDVSSGKCLKTLKGHSNYVFCCNFNPQSNLIVSGSFDESVRIWDVKTGKCLKTLPAHSD
PVSAVHFNRDGSLIVSSSYDGLCRIWDTASGQCLKTLIDDDNPPVSFVKFSPNGKYILAATLDNTLKLWDYSKGKCLKTYTGHKNEK
YCIFANFSVTGGKWIVSGSEDNLVYIWNLQTKEIVQKLQGHTDVVISTACHPTENIIASAALENDKTIKLWKSDC

Vector:p28aLIC

Growth

Medium:
Antibiotics:
Procedure:WDR5 was expressed in E. coli BL21 (DE3) Codon Plus RIL in Terrific Broth (TB) in the presence of 50 µg/ml of kanamycin. Cell were grown at 37 oC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside (IPTG), final concentration 1 mM, and incubated overnight at 12 degC.

Purification

Procedure
Column 1: 5 ml HiTrap Chelating column (Amersham Biosciences)
Column 2: Superdex200 column (26x60) (Amersham Biosciences)

The crude extract was cleared by centrifugation. 5 mM imidazole was added to the lysate. The sample was loaded onto 5 ml HiTrap Chelating column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM Hepes-NaOH buffer, pH 7.5, containing 500 mM NaCl and 50 mM imidazole, and the protein was eluted with elution buffer. 10 mM DTT

was added to WDR5 containing fractions. The protein was loaded on Superdex200 column (26x60) (Amersham Biosciences), equilibrated with 20 mM Hepes-NaOH buffer, pH 7.5, and 500 mM NaCl, at flow rate 4 ml/min. Thrombin (Sigma) was added to combined fractions containing WDR5 and incubated overnight at 4°C. Since the cleavage was not complete the sample was further purified to homogeneity by another affinity chromatography on Ni HisTrap (1 mL) (Amersham Biosciences). Purification yield is 98 mg of protein per 1L of culture.

Extraction

Procedure
Cells were harvested by centrifugation at 7,000 rpm. The cell pellets were frozen in liquid nitrogen and stored at -80 degC. For the purification the cell paste was thawed and resuspended in lysis buffer with protease inhibitor (0.1 mM phenylmethyl sulfonyl fluoride, PMSF). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 18,000 psi.
Concentration:28.9 mg/ml
Ligand
MassSpec:expected mass = 36732.6 Da, measured mass = 36733.4 Da
Crystallization: Purified WDR5 was crystallized using the sitting drop vapor diffusion method at 18 °C by mixing 1 µl of 10 mg/mL protein solution with 1 µl of the reservoir solution containing 20 % PEG 5000 MME and 0.1 M BisTris pH 6.5.
NMR Spectroscopy:
Data Collection:
Data Processing: