WDR5

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Revision

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Entry Clone Accession:GI:16554627
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SGC Clone Accession:WDR5:APC041-H09:C36428
Tag:N-terminal: His-tag with integrated TEV protease site:MHHHHHHSSGRENLYFQG
Host:E.coli BL21 (DE3) codon plus RIL (Stratagen).

Construct

Prelude:
Sequence:

gTQSKPTPVKPNYALKFTLAGHTKAVSSVKFSPNGEWLASSSADKLIKIWGAYDGKFEKTISGHKLGISDVAWSSDSNLLVSASDDK
TLKIWDVSSGKCLKTLKGHSNYVFCCNFNPQSNLIVSGSFDESVRIWDVKTGKCLKTLPAHSDPVSAVHFNRDGSLIVSSSYDGLCR
IWDTASGQCLKTLIDDDNPPVSFVKFSPNGKYILAATLDNTLKLWDYSKGKCLKTYTGHKNEKYCIFANFSVTGGKWIVSGSEDNLV
YIWNLQTKEIVQKLQGHTDVVISTACHPTENIIASAALENDKTIKLWKSDC

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:WDR5 was expressed in E.coli BL21 (DE3)codon plus RIL in TB medium in the presence of 50 µg/ml of kanamycin. Cell weregrown at 37oC to an OD600 of 1.5 and induced by isopropyl-1-thio-D-galactopyranoside(IPTG), final concentration 1 mM and incubated overnight at 15oC.

Purification

Procedure
The crude extract was cleared by centrifugation. The clarifiedlysate was loaded onto 5 ml HiTrap Chelating column (Amersham Biosciences), chargedwith Ni2+. The column was washed with 10 CV of 20 mM HEPES, pH 7.4, containing250 mM NaCl, 50 mM imidazole, 5% glycerol, and the protein was eluted with elutionbuffer (20 mM HEPES pH 7.4, 250 mM NaCl, 250 mM imidazole, 5% glycerol).The protein was loaded on Superdex200 column (26x60) (Amersham Biosciences),equilibrated with 20 mM PIPES buffer, pH 6.5, and 250 mM NaCl, at flow rate 4 ml/min. The fractions containing WDR5 were pooled and treated with TEV protease toremove

His-Tag. The protein was further purified to homogeneity by ion-exchangechromatography on Source 30S column (10x10) (Amersham Biosciences), equilibratedwith buffer 20 mM PIPES, pH 6.5, and eluted with linear gradient of NaCl up to 500 mMconcentration (20CV). Purification yield was 11 mg of the protein per 1L of culture.

TEV cleavage.

Extraction

Procedure
Cells were harvested by centrifugation at 12,227 Xg. The cell pellets were frozen in liquid nitrogen and stored at -80˚C. For thepurification, 11 g of the cell paste was thawed and resuspended in 110 ml lysis buffer(50 mM HEPES, pH 7.4, 250 mM NaCl, 5 mM imidazol, 2 mM β-mercaptoethanol, 5%glycerol) with protease inhibitor (1mM phenylmethyl sulfonyl fluoride, PMSF). Thecells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.
Concentration:18 mg/ml
Ligand
MassSpec:characterization: expected MW = 34245.9 Da, measured MW= 34248.5767Da.
Crystallization: Purified WDR5 (10 mg/mL) was complexed with compound at 1:5molar ratio of protein:compound and crystallized using the sitting drop vapor diffusionmethod at 20 °C by mixing 1 µl of the protein solution with 1 µl of the reservoir solutioncontaining 22% PEG3350, 0.1M NH_4SO_4, 0.1 M BisTris, pH 6.0.
NMR Spectroscopy:
Data Collection:
Data Processing: