

WDR5

PDB:4IA9

Revision

Revision Type:created

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Entry Clone Accession:GI:16554627

Entry Clone Source:MGC

SGC Clone Accession:

Tag:N-terminal: His-tag with integrated TEV protease site: MHHHHHHSSGRENLYFQG

Host:E.coli BL21 (DE3) codon plus RIL (Stratagen).

Construct

Prelude:

Sequence:

gTQSKPTPVKP NYALKFTLAGHTKAVSSVKFSPNGEWLASSSADKLIKIWGAYDGKFEKTISGHKLGISDVAWSSDSNLLVSASDDK
TLKIWDVSSGKCLKTLKGHSNYVFC CNFNPQSNLIVSGSFDES VRIWDVKTGKCLKTLPAHSDPVSAVHFNRDGS LIVSSSYDGLCR
IWDTASGQCLKTLIDDDNPPVSFVKFSPNGKYILAATLDNTLKLWDYSKGKCLKTYTGHKNEKYCIFANFSVTGGK WIVSGSEDNLV
YIWNLQTKEIVQKLQGHTDV VISTACHPTENIIASAALENDKTIKLWKS D C

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 were grown 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 clarified lysate was loaded onto 5 ml HiTrap Chelating column (Amersham Biosciences), charged with Ni2+. The column was washed with 10 CV of 20 mM HEPES, pH 7.4, containing 250 mM NaCl, 50 mM imidazole, 5% glycerol, and the protein was eluted with elution buffer (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 to remove

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

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 the purification, 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). The cells were lysed by passing through Microfluidizer (Microfluidics Corp.) at 20,000 psi.

Concentration: 18 mg/ml - Enzymatic treatment: TEV cleavage.

Ligand

MassSpec: expected MW = 34245.9 Da, measured MW = 34248.5767 Da.

Crystallization: Purified WDR5 (10 mg/mL) was complexed with compound SGC-DS-MT-0221-b1 at 1:5 molar ratio of protein:compound and crystallized using the sitting drop vapor diffusion method at 20 °C by mixing 1 μ l of the protein solution with 1 μ l of the reservoir solution containing 25% PEG3350, 0.1M NH4oAC, 0.1 M BisTris, pH 5.5.

NMR Spectroscopy:

Data Collection:

Data Processing: