NSD2-PWWP1 Purification

Clone sample ID: WHSC1:MVC008-C10:C240432

Construct ID: Bio1_WHSC1-32

Vector: p28BIOH-LIC

N-terminal tag: msglndifeaqkiewhegsaggsg **C-terminal tag:** ggsghhhhhh

Residues (start- end): 208- 368

msglndifeaqkiewhegsaggsgPNTGRDKDHLLKYNVGDLVWSKVSGYPWWPCMVSADPLLHSYT
KLKGQKKSARQYHVQFFGDAPERAWIFEKSLVAFEGEGQFEKLCQESAKQAPTKAEKIKLLKPI
SGKLRAQWEMGIVQAEEAASMSVEERKAKFTFLYVGDQLHLNPQVAKEAGIAAEggsghhhhhh

Host: *E. Coli, BL21 (DE3),* containing BirA Ligase

Yield: 5 mg/L

Storage buffer: 20 mM Tris, pH 8.0, 500 mM NaCl, 1 mM TCEP, 5% Glycerol

Purity uncut protein: 95%

Construct and Expression:

DNA fragment encoding human WHSC1 (residues 208- 368) was amplified by PCR and sub-cloned into p28BIOH-LIC vector, downstream of an AviTag and the upstream of a poly-histidine coding region. Following transformation into *E. Coli* BL21 (DE3) the cells were amplified at 37°C by inoculating Terrific Broth with overnight culture, both supplemented with 50 µg/ml Kanamycin and 35 µg/ml chloramphenicol. When the OD600 of the culture reached 0.8- 1.5, the temperature was lowered to 18°C and the target protein was over-expressed by inducing cells with 0.5 mM IPTG (isopropyl-1-thio-D-galactopyranoside) and D-Biotin was added at 10 µg/mL final concentration and incubated overnight before being harvested (7000 rpm for 10 min at 4˚C) using a Beckman Coulter centrifuge.

Harvest and cell lysis:

Harvested cells were re-suspended in 20 mM Tris-HCl, pH 7.5 , 500 mM NaCl, 5 mM imidazole and 5% glycerol, 1X protease inhibitor cocktail (100 X protease inhibitor stock in 70% ethanol (0.25 mg/ml Aprotinin, 0.25 mg/ml Leupeptin, 0.25 mg/ml Pepstatin A and 0.25 mg/ml E-64) or Pierce™ Protease Inhibitor Mini Tablets, EDTA-free. The cells were lysed chemically by rotating 30 min with 0.5% CHAPS, 1 mM TCEP, 1 mM PMSF and 5/L µL Benzonase Nuclease (In-House) followed by sonication at frequency of 8 (5” on/7” off) for 5 min (Sonicator 3000, Misoni). The crude extract was clarified by high-speed centrifugation (60 min at 36,000 ×g at 4˚C) by Beckman Coulter centrifuge.

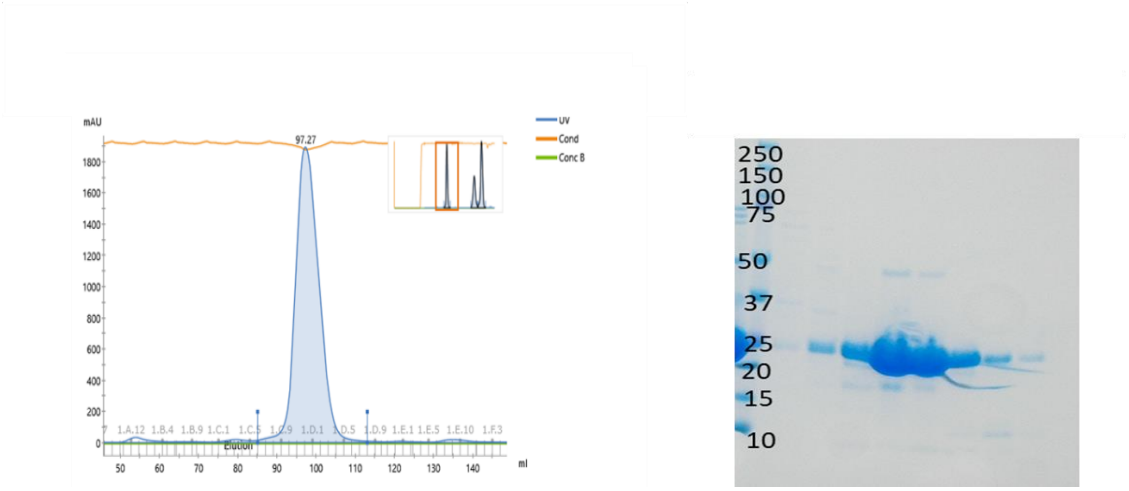
Purification columns:

- Ni-NTA
- Superdex200 16/60

Purification SOP:

The clarified lysate was then loaded onto an open column containing pre-equilibrated Ni-NTA (Qiagen). The column was washed with 20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 5% glycerol, 5 mM imidazole, then with 1 mM D-Biotin in PBS, followed by 20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 5% glycerol, 20 mM imidazole. Finally, the protein was eluted by running 20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 5% glycerol, 250 mM imidazole. The eluted protein was then supplemented with 1mM TCEP and concentrated to be further purified by gel filtration on a HiLoad Superdex200 16/600 using an ÄKTA Pure (GE Healthcare). The gel filtration column was pre-equilibrated with 20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 1 mM TCEP, 5% glycerol. The purity of the fractions was assessed on SDS-PAGE gel and pure fractions were pooled, concentrated and flash frozen.

Gel Filtration Chromatography: Superdex200 16/600



Expected size: 21766.63 Da + 226 Da Biotin addition = 21992.63 Da OR <u>21861.44 Da (considering cleavage of 1st Methionine)</u>

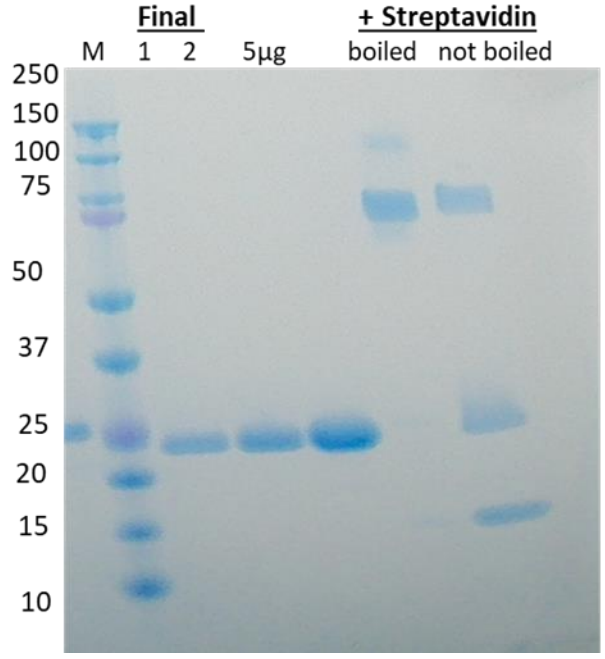
Mass Spec: 21861.88 Da

The Gel Shift Assay:

The gel shift assay was performed to determine the relative extent and efficiency of biotinylation of the recombinant protein. Briefly, 10 µg of the protein was mixed with 20 µl of streptavidin (1 mg/mL) and the mixture was further diluted to a 50 µl final volume with 20 mM Tris buffer, pH 7.5. The mixture was stored at room temperature for 30 min. Then, 4X sample buffer including reducing agent was added. At this point, the sample was divided into two parts. First part was loaded on the SDS-PAGE gel (10 µL) without boiling (non-denaturing condition, NDC) and the second part was loaded after boiling at 95-100 °C for 5 min (denaturing condition, DC). The gel was run under appropriate voltage and amount of time and was stained with Coomassie blue. The level of biotinylation is then measured by comparing the intensity of the shifted band of the desired protein in the presence of streptavidin under NDC.

Shift Assay: >90%

Purity assessment: SDS-PAGE on 4-12% Bis-Tris Gel (Life technology)



Lane 1: Precision Plus Protein standard, Bio-Rad

Lanes 2-4: 1, 2 and 5 µg NSD2 after Gel filtration

Lane 5: WHSC1L1 + Streptavidin, ‘denatured, boiled’

Lane 6: WHSC1L1 + Streptavidin, ‘native, non-boiled’