Written By: Elisa Gibson
Reviewed By: Taraneh Hajian

Date: 2020-10-20
Version 1

Clone sample ID: WHSC1:JMC027-A07:C212867

Construct ID: WHSC1-32

Vector: pET28-MHL

N-terminal tag: Mhhhhhhssgrenlyfqg **C-terminal tag:** N/A

Residues (from-to): 208- 368

Uncut:
MhhhhhhssgrenlyfqgPNTGRDKDHLLKYNVGDLVWSKVSGYPWWPCMVSADPLLHSYTKLKG
QKKSARQYHVQFFGDAPERAWIFEKSLVAFEGEGQFEKLCQESAKQAPTKAEKIKLLKPISGKL
RAQWEMGIVQAEEAASMSVEERKAKFTFLYVGDQLHLNPQVAKEAGIAAE

Cut:
gPNTGRDKDHLLKYNVGDLVWSKVSGYPWWPCMVSADPLLHSYTKLKGQKKSARQYHVQFF
GDAPERAWIFEKSLVAFEGEGQFEKLCQESAKQAPTKAEKIKLLKPISGKLRAQWEMGIVQAEE
AASMSVEERKAKFTFLYVGDQLHLNPQVAKEAGIAAE

Host: *E. Coli, BL21 (DE3)*

Yield: >10 mg/L

Storage buffer: 20 mM Tris-HCl pH 7.4, 500 mM NaCl, 5% Glycerol

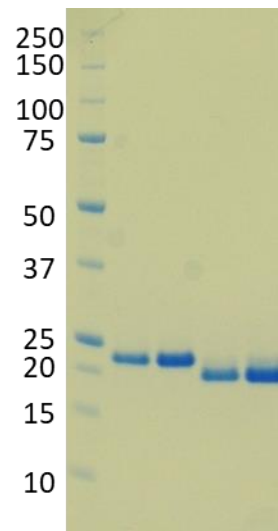
Purity cut and uncut protein: >95%

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

Lane 1: Precision Plus Protein standard, Bio-Rad

Lanes 2- 3: 1 and 2 µg uncut

Lanes 4- 5: 1 and 2 µg cut



SGC

Molecular Biophysics

Construct and Expression:

DNA fragment encoding human NSD2 (residues 208- 368) was amplified by PCR and sub-cloned into a pET28-MHL vector, downstream of the 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 16°C and the target protein was over-expressed by inducing cells with 0.5 mM IPTG (isopropyl-1-thio-D-galactopyranoside) 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 15 µL Benzonase Nuclease (In-House) followed by sonication at frequency of 7 (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:

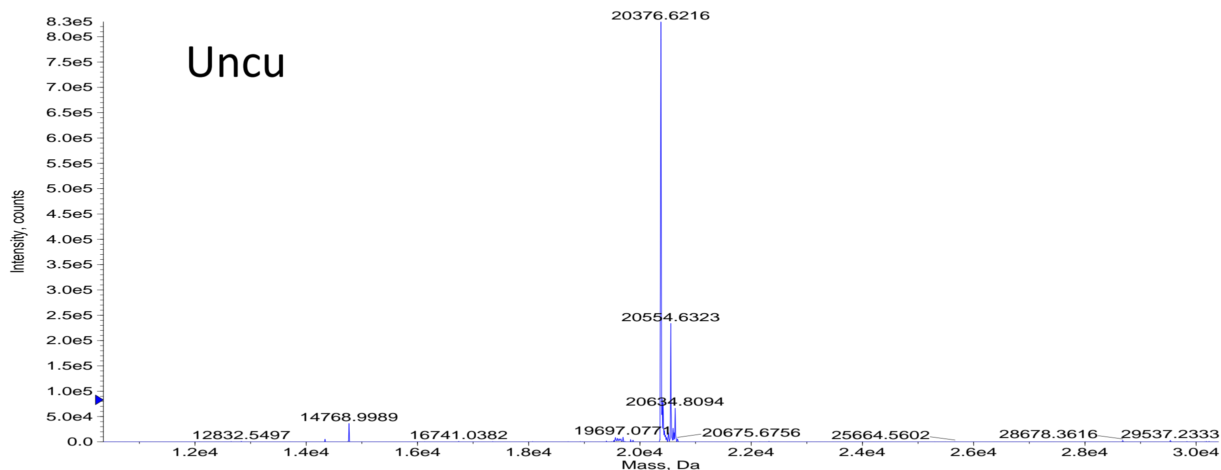
The clarified lysate was then loaded onto an open column containing pre-equilibrated Ni-NTA (Qiagen). The column was washed and eluted by running 20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 5% glycerol, containing 5 mM, 20 mM, and 250 mM imidazole, respectively. The eluted protein was analyzed for purity by SDS-PAGE. Since it was quite pure after Ni-NTA, the protein was split into two portions for dialysis overnight in 20 mM Tris-HCl pH 7.4, 500 mM NaCl, 5% glycerol. One portion had TEV protease added to facilitate tag cleavage. This portion was subjected to a second Ni-NTA column to remove TEV, tag and uncut protein. After tag removal, both uncut and cut WHSC1 was concentrated, aliquoted and flash frozen.

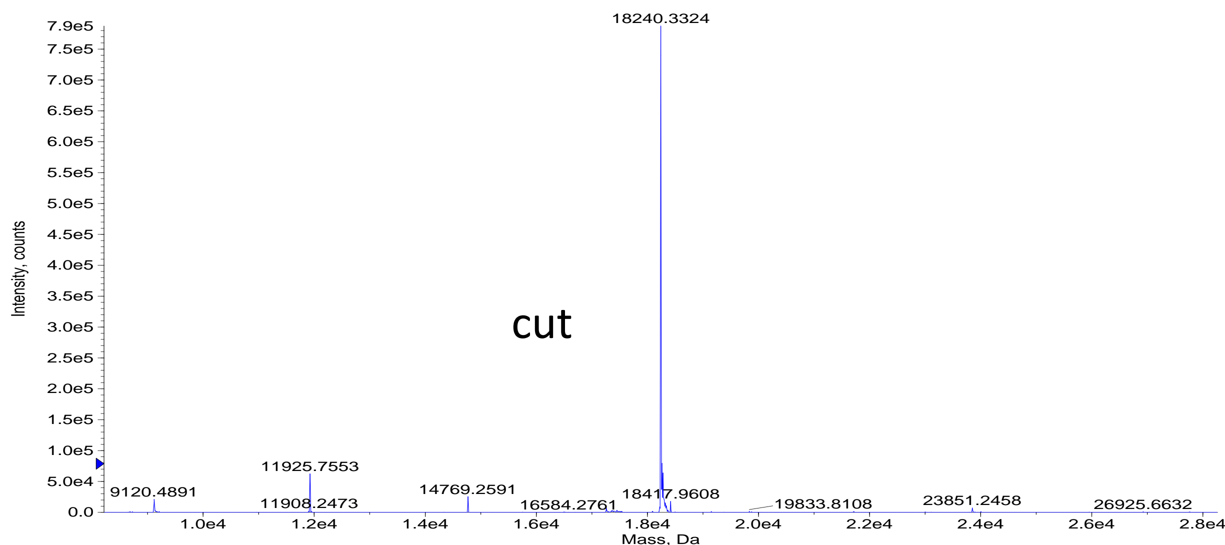
Expected size Uncut: 20376.21 Da

Mass Spec Uncut: 20376.62 Da

Expected size Cut: 18239.91 Da

Mass Spec Cut: 18240.33 Da





SGC

Molecular Biophysics