Clone sample ID: FBXW7:MVC011-B06:C242774

Construct ID: FBXW7_349-707-BV

Vector: pFBOH-MHL

N-terminal tag: MHHHHHHSSGRENLYFQG

Residues (start-end): 349-707

MHHHHHHSSGRENLYFQGSPWKSAYIRQHRIDTNWRRGELKSPKVLKGHDDHVITCLQFCGN
RIVSGSDDNTLKVWSAVTGKCLRTLVGHTGGVWSSQMRDNIIISGSTDRTLKVWNAETGECIH
TLYGHTSTVRCMHLHEKRVVSGSRDATLRVWDIETGQCLHVLMGHVAAVRCVQYDGRRVVS
GAYDFMVKVWDPETETCLHTLQGHTNRVYSLQFDGIHVVSGSLDTSIRVWDVETGNCIHTLTG
HQSLTSGMELKDNILVSGNADSTVKIWDIKTGQCLQTLQGPNKHQSAVTCLQFNKNFVITSSDD
GTVKLWDLKTGEFIRNLVTLESGGSGGVVWRIRASNTKLVCAVGSRNGTEETKLLVLDFDVDM
K

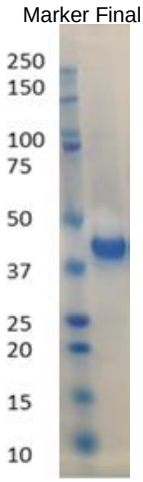
Host: sf9

Purity protein: 95%

Yield: 16 mg/L

Storage buffer: 50 mM Tris 8, 150 mM NaCl, 5% Glycerol ,5 mM 2-Mercaptoethanol

SDS-PAGE gel on 4-12% Bis-Tris Gel (Life technology) with 250 kDa Bio-Rad Precision Plus Dual colored:



Construct and Expression

DNA fragments encoding the human FBXW7 residues 349-707 amplified by PCR and sub-cloned into pFBOH-MHL downstream of the HisTag. The resulting plasmid was transformed into DH10Bac™ Competent E. coli (Invitrogen) and a recombinant viral DNA bacmid was purified and followed by a recombinant baculovirus generation in s9 insect cells. sf9 cells grown in HyQ® SFX insect serum-free medium (Thermo Scientific) were infected with 10 ml of P3 viral stock per 0.8L of suspension cell culture and incubated at 27°C using a platform shaker set at 100RPM.The cells were collected after 72 hours of post infection time, when viability dropped to 70-80%.

Harvest and cell lysis

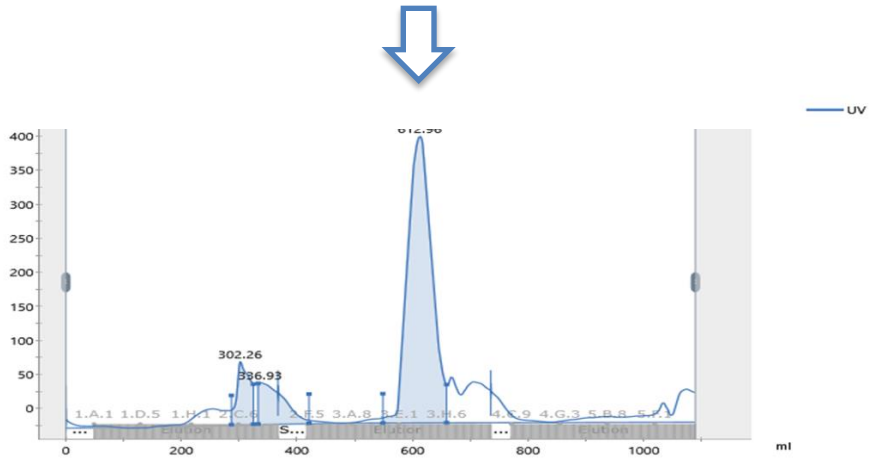
Harvested cells were re-suspended in 20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 5 mM imidazole and 5% glycerol, 1X protease inhibitor cocktail (100 X protease inhibitor stock in 70% ethanol (0.25 mg/ml Aprotinin, 0.25 mg/ml Leupeptin, 0.25 mg/ml Pepstatin A and 0.25 mg/ml E-64) or Roche complete EDTA-free protease inhibitor cocktail tablet. The cells were lysed chemically by rotating 30 min with NP40 (final concentration of 0.6%), 120 µl/L Benzonase nuclease (in house) followed by sonication at frequency of 7.5 (10” on/10” 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

Purification columns:

- Ni-NTA resin
- Superdex200 26/600

Clarified lysate was passed through Ni-NTA resin (Qiagen) column. The column was washed by running 20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 5% glycerol, containing 30 mM imidazole and eluted by same buffer containing 250 mM imidazole. The protein was then purified further by gel filtration on a Superdex200 26/600 using an AKTA Pure (GE Healthcare), pre-equilibrated in 50 mM Tris-HCl 8.0, 150 mM NaCl, 5% Glycerol ,5 mM 2-Mercaptoethanol. The purity of the fractions was confirmed on a SDS-PAGE gel and the pure fractions were pooled and flash frozen.



Expected size: 42086.64 Da

Observed Mass: 42129.80 Da