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USP5 Zf-UBD Expression & Purification

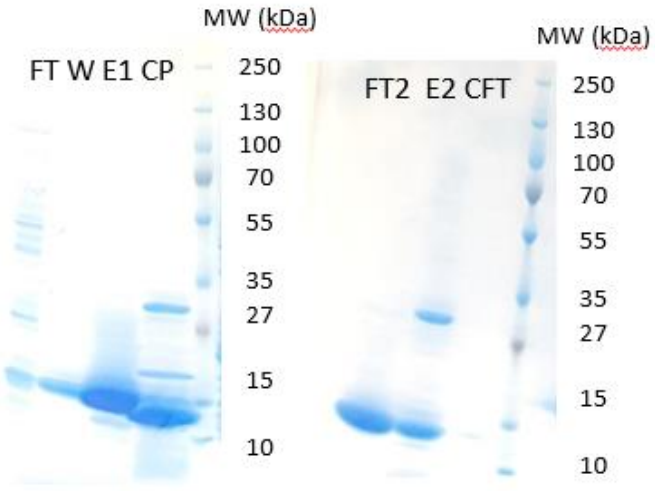
Growth:

3x2 L M9 minimal media culture in BL21 Codon Plus RIL grown at 37˚C in shaker, induced at OD_{600} ~0.55 with 0.5 mM IPTG. Cultures grown overnight at 15˚C.

Construct ID	Vector	Cloned AA Sequence	MW	pI
SDC132:E06	pET28-MHL	GEVRQVSKHAFSLKQLDNPARIPPCGWKCSKC DMRENLWLNLTDGSILCGRRYFDGSGGNNHA VEHYRETGYPLAVKLGTITPDGADVYSYDEDD MVLDPSLAEHLSHFGIDMLKMQKTD	13460.13	5.42

Purification:

Cells were harvested by centrifugation. Cells were re-suspended in 400 mL of resuspension buffer 50 mM Tris pH 8, 150 mM NaCl, 2 mM TCEP supplemented with benzonase and 1x protease inhibitors and lysed by sonication. Clarified lysate (supernatant-SN) rocked with 5 mL Ni-NTA resin for 1 hour at 4˚C (flow through-FT). Beads washed with 100 mL resuspension buffer, then 200 mL resuspension buffer + 15 mM imidazole (Wash 2-W) before elution with 2x15 mL resuspension buffer + 300 mM imidazole (elution-E1). Eluent supplemented with 100 U of thrombin and dialysed with snakeskin MWCO 3500 against 1 L re-suspension buffer (no imidazole) for 2 hours then fresh 2 L resuspension buffer overnight (cleaved protein-CP). Cleavage of protein was verified with SDS-PAGE analysis. Cleaved protein incubated with 5 mL Ni resin and rocked for 1 hour at 4˚C (FT2). The beads were eluted with dialysis buffer + 300 mM imidazole (E2). FT2 of USP5 concentrated to 5 mL and run on S75 16/60. Concentrator flow through (CFT).



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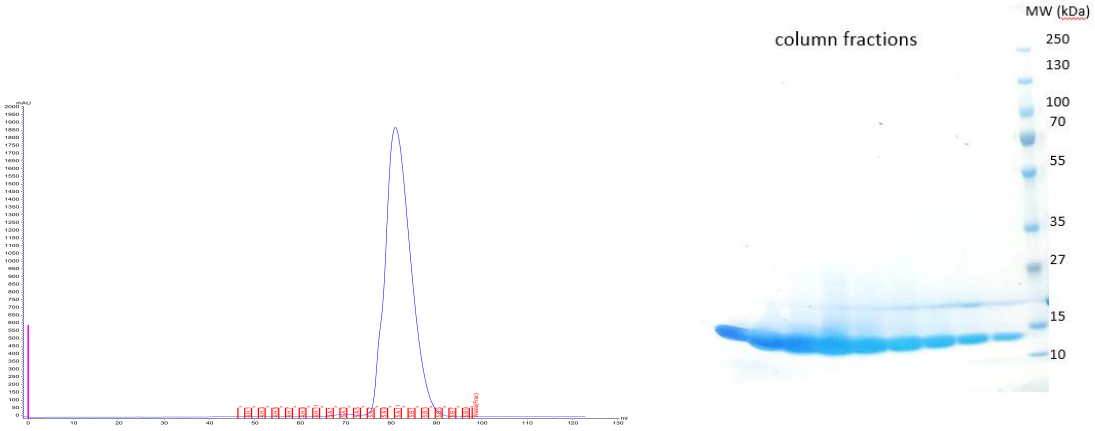

Fractions were concentrated to 8.3 mg/mL and aliquoted (50 µL x 31), frozen in liquid N2 and stored at -80˚C. The final yield was 12.8 mg. Mass spectrometry was used to verify purity; the correct mass of the construct was observed.