Purification of ZZ-tev-PP7-His from E. coli
Protocol from Bobby Hogg

Transform pET28ZZTPP7H into BL21 (DE3) cells. Plate on LB-Kan (50ug/mL) plates. Pick a single colony to inoculate a 5mL starter culture with 25 μg/mL Kan. Grow overnight at 37°C.

Dilute starter culture 1:500 into 500 mL 2xYT + 25 μg/mL Kan. Grow at 37°C to O.D. 0.4-0.5. Induce with 1 mM IPTG. Grow at 37°C for 4 hours. Harvest cells. Can continue with prep or freeze cell pellets at -20°C.

Resuspend pellet from 500 mL culture in 20 mL: 20 mM Tris pH 8
1 mM $MgCl_2$
10% Glycerol
Protease inhibitors (PMSF, aprotinin, leupeptin, pepstatin)

Sonicate 3 x 30 seconds.

Add: 0.1% NP-40
200 mM NaCl
20 mM Imidazole

Centrifuge 12.5K rpm in SS-34 rotor for 20 minutes, 4°C.

Add supernatant to 0.5 mL Qiagen Ni-NTA resin washed 3x into Wash Buffer.

Bind 60 minutes, 4°C.

Spin gently in clinical to pellet beads. Remove majority of sup, use remainder to pack Bio-Rad 10 mL Poly-Prep column.

Wash with 20 mL Wash Buffer: 20 mM Tris pH 8
1 mM $MgCl_2$
200 mM NaCl
20 mM Imidazole
0.1% NP-40
10% Glycerol
Protease Inhibitors

Elute 3x 800 μL Wash Buffer + 250 mM Imidazole
Aliquot and snap freeze in liquid nitrogen.
Monitor concentration and purity by Bradford and SDS-PAGE.