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## Native-PAGE analysis of VCP hexamer

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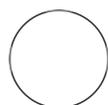
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The AAA+ chaperone VCP disaggregates Tau fibrils and generates aggregate seeds  
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Felix Kraus

### ABSTRACT

Valosin-containing protein (VCP) is a homo-hexameric AAA+ ATPase in eukaryotic cells. This protocol describes the analysis of myc-tagged versions of VCP transiently transfected in HEK293 cells (stably expressing and propagating aggregates of Tau repeat domain fused to YFP) for hexamer formation.

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We use this protocol and it's working

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- 1** Plate  $1.5 \times 10^5$  cells in 12-well plate.
- 2** Next day, transfect with plasmids expressing myc-tagged VCP variants (Saha et al. BioRxiv, 2022) using a standard transfection protocol.
- 3** Two days later, collect cells and lyse them in 50  $\mu$ L 0.5% Triton X-100/PBS supplemented with protease inhibitor cocktail (Roche) and DNase for 1 h on ice. 1h
- 4** Centrifuge lysates at 10,000 x g for 2 min and collect supernatant. 2m
- 5** Determine protein concentration in the supernatant and normalize across all samples.

**6** Add 2x native sample buffer (40 % glycerol, 240 mM Tris pH 6.8, 0.04 % bromophenol blue) to 40 µg lysate.

**7** Run samples on a Native PAGE gel (e.g. Novex Value 4 to 12% Tris-glycine gels (Thermo)) in 20 mM Tris 200 mM Glycine buffer at pH 8.4. 1h

**8** Transfer proteins to nitrocellulose membrane in standard Tris-glycine buffer, block in 5% low-fat dry milk for 1 h at room temperature (RT). 2h

**Note**

NOTE: Nitrocellulose membranes produce less background than PVDF membranes with fluorescent secondary antibodies.

**9** Dilute anti-myc (9E10) and anti-VCP (1:2000, Novus Biologicals) primary antibodies together in blocking solution and incubate membrane overnight.

**10** Next day, wash membrane 3 times with TBST and incubate with anti-mouse (LI-COR Biosciences Cat# 926-68070, RRID:AB\_10956588; 1:10,000) and anti-rabbit (LI-COR Biosciences Cat# 926-32211, RRID:AB\_621843; 1:10,000) fluorescent secondary antibodies for 2 h at RT. 2h

**11** Wash membrane 3 times with TBST.

**12** Detect fluorescent myc and VCP signal on a fluorescent imager.