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Native-PAGE analysis of VCP hexamer

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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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The AAA+ chaperone VCP disaggregates Tau fibrils and generates aggregate seeds Itika Saha, Patricia Yuste-Checa, Miguel Da Silva Padilha, Qiang Guo, Roman Körner, Hauke Holthusen, Victoria A. Trinkaus, Irina Dudanova, Rubén Fernández-Busnadiego, Wolfgang Baumeister, David W. Sanders, Saurabh Gautam, Marc I. Diamond, F. Ulrich Hartl, Mark S. Hipp bioRxiv 2022.02.18.481043; doi: https://doi.org/10.1101/2022.02.18.481043

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1 Plate 1.5x105 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 µL 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

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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: 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.

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