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Detection of Tau ubiquitylation

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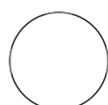
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The AAA+ chaperone VCP disaggregates Tau fibrils and generates aggregate seeds
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ABSTRACT

This protocol describes the detection of ubiquitylated Tau from HEK293 cells stably expressing and propagating aggregates of Tau repeat domain fused to YFP (Sanders et al. Neuron, 2014; Saha et al, BioRxiv, 2022).

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Protocol status: Working
We use this protocol and it's working

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- 1 Harvest cells from 2 confluent wells of a 6 well plate.
- 2 Lyse aggregate-containing cells by vortexing in cold RIPA buffer (Thermo) supplemented with protease inhibitor cocktail (Roche) and DNase. 20 mM N-ethylmaleimide should be included in the lysis buffer to inhibit the activity of deubiquitinating enzymes.
- 3 Briefly sonicate lysate and centrifuge at 2,000 x g for 5 min to remove cell debris. 5m
- 4 Collect supernatant, determine protein concentration and normalize across samples.
- 5 Dilute 1 mg protein in a total volume of 600 μ L RIPA buffer.

- 6 Add 50 μ L anti-GFP bead slurry (μ MACS GFP Isolation kit, Miltenyi Biotec) to diluted lysate.
- 7 Incubate for 1 h at 4 °C in a rotating wheel at 10 rpm. 1h
- 8 Before the end of 1 h, place μ -columns (Miltenyi Biotec) in the magnetic field of a μ MACS Separator (Miltenyi Biotec) and equilibrate columns by applying 250 μ L RIPA buffer. Allow complete flow-through.
- 9 Apply cell lysates and beads to μ -columns. Allow complete flow-through.
- 10 Wash columns 4 times with 1 mL 0.1% SDS/PBS.
- 11 Elute by applying 50 μ L pre-heated (95 °C) 1x SDS sample buffer.
- 12 Analyze eluates by immunoblotting with antibodies against GFP or ubiquitin.