Detection of Tau ubiquitylation

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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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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-ethylmalemide 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 µL RIPA buffer.

6 Add 50 µL anti-GFP bead slurry (µMACS GFP Isolation kit, Miltenyi Biotec) to diluted lysate.

7 Incubate for 1 h at 4 °C in a rotating wheel at 10 rpm. 1h

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8 Before the end of 1 h, place µ-columns (Miltenyi Biotec) in the magnetic field of a µMACS Separator (Miltenyi Biotec) and equilibrate columns by applying 250 µL 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 µL pre-heated (95 °C) 1x SDS sample buffer.

12 Analyze eluates by immunoblotting with antibodies against GFP or ubiquitin.

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