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# Biochemical detection of aggregated Tau

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**We use this protocol and it's working**

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## Abstract

This protocol describes the detection of aggregated 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). The filter trap assay is a modification of the membrane filter assay described previously (Wanker et al., Methods in Enzymology, 1999).

## Troubleshooting

## Preparation of cell lysates

1 The protocol can be performed with freshly harvested cells or with frozen cell pellets. In either case, harvest cells by trypsinization and wash 1x with PBS before pelleting in 1.5 mL Eppendorf tubes.

2 Thaw frozen cell pellets on ice for at least 10 min. 10m

3 For cell pellets harvested from 1 well of a 12-well plate, add 75 µL RIPA lysis buffer (Thermo) or 1% Triton X-100/PBS supplemented with protease inhibitor cocktail (Roche) and DNase, and resuspend pellets by pipetting.

4 When using 1% Triton X-100/PBS, briefly sonicate samples to lyse nuclei and achieve a homogenous lysate.

5 Incubate on ice for 30 min to 1 h. 1h

6 Centrifuge lysates at 1,000 x g for 5 min at 4 °C. Carefully remove supernatant without disturbing pelleted debris. 5m

> Note
>
> NOTE: Debris in the lysate can clog the filter in a filter trap assay.

7 Quantify protein concentration and normalize across all samples. The lysate can now be subjected to the centrifugation-based solubility assay or filter trap assay described below.

## Solubility assay

8 Centrifuge lysate at 186,000 x g for 1 h at 4 °C. 1h

9 Remove supernatant and wash pellet with 200 µL PBS.

10 Centrifuge at 186,000 x g for 30 min at 4 °C. 30m

11 Remove PBS.

12 Add the same volume of PBS to the pellet as the lysis buffer initially used for the assay.

13 Disintegrate pellet by pipetting. Alternatively, sonication can be used.

14 Dilute total lysate, supernatant and pellet with SDS sample buffer and analyze by immunoblotting.

## Filter trap assay

15 Equilibrate cellulose acetate membrane in 0.1% SDS/H2O until completely wet.

16 Dilute up to 200 µg (Triton) or 100 µg (RIPA) total lysate in a total volume of 200 µL pre-cooled lysis buffer.

17 Mix samples by gentle vortexing and spin down in a mini centrifuge.

18 Affix equilibrated membrane to a filter trap apparatus (e.g. (PR648 Slot Blot Blotting Manifold, Hoefer). Ensure that there are no bubbles trapped over the membrane.

19 Load 200 µL 0.1% SDS/H2O to the wells and apply vacuum. Observe whether the liquid is completely drawn through.

20 Load lysates and wait until they completely pass through the filter.

Note

NOTE: Filter clogging produces faulty results.

21 Wash wells 3 times with 200 µL 0.1% SDS/H2O followed by standard immunoblotting of the membrane.