

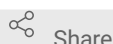


Sep 06, 2022

Size exclusion chromatography of cell lysates containing Tau aggregates

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Felix Kraus

ABSTRACT

This protocol can be used to fractionate Tau aggregates from cell lysates by size exclusion chromatography. The protocol was optimized using HEK293 cells stably expressing and propagating aggregates of Tau repeat domain, containing the disease-related mutations P301L and V337M, fused to YFP (Clone 10 from Sanders et al., Neuron, 2014). However, any cell line containing Tau aggregates can be used.

DOI

dx.doi.org/10.17504/protocols.io.4r3l27mjpg1y/v1

EXTERNAL LINK

<https://www.biorxiv.org/content/10.1101/2022.02.18.481043v1.full>

PROTOCOL CITATION

Patricia Yuste Checa, Itika Saha, F. Ulrich Hartl, Mark S. Hipp 2022. Size exclusion chromatography of cell lysates containing Tau aggregates. **protocols.io** <https://protocols.io/view/size-exclusion-chromatography-of-cell-lysates-containing7xtrpn>

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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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CREATED

Sep 05, 2022

LAST MODIFIED

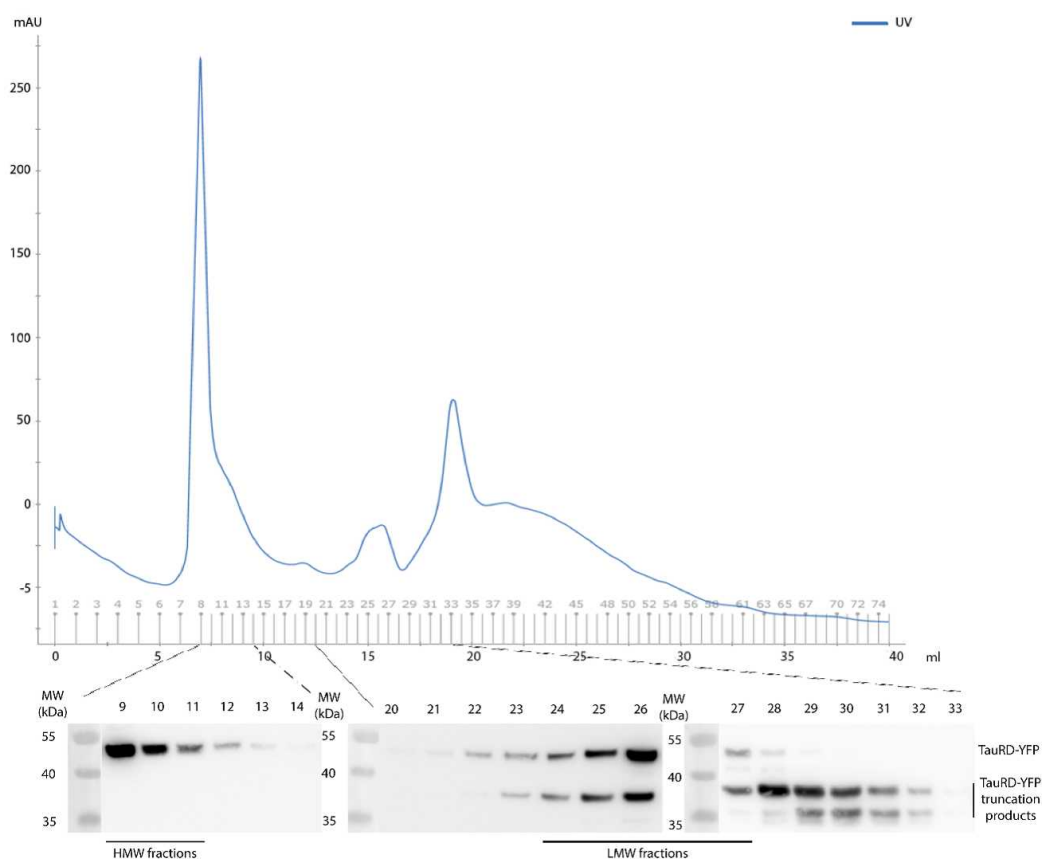
Sep 06, 2022

PROTOCOL INTEGER ID

69591

- 1 Lyse cell pellets with Triton buffer, Complete EDTA-free protease inhibitor cocktail (Roche) and ^{30m}benzonase for 20 min on ice. NOTE: Triton buffer: 0.05% Triton X-100/PBS or 1% Triton X-100/PBS (more efficient lysis) can be used to lyse cell pellets.
- 2 Clarify the lysates by centrifugation at 1,000 x g for 5 min at 4 °C and filter the supernatant with a ^{5m}PVDF 0.22 µm filter (Millex, #SLGVX13NL).
- 3 Quantify total protein by Bio-Rad Protein Assay (Bio-Rad) or Pierce BCA Protein Assay Kit (Thermo Fisher Scientific).
- 4 Load 3 mg total protein on a Superose 6 HR10/30 (GE 875 Healthcare) column previously equilibrated with PBS.
 - 4.1 NOTES: Cells from a confluent T75 flask lysed with 800 µL lysis buffer results in ~ 8-10 mg/mL total protein. Superose 6 HR10/30 (GE Healthcare): Flow rate 0.5 mL/min; 1mL fractions up to void volume of the column and 0.5 mL fractions from void volume to the end.
- 5 Analyze and quantify individual fractions by Western blot. NOTES: Use 15 µl of each fraction to detect TauRD-YFP by immunoblotting with anti-GFP antibody (Roche #11814460001).

5.1



High molecular weight (HMW) species are detected in the void volume of the column while low molecular weight (LMW) species are detected around 15 mL elution volume (fractions ~24-27). TauRD-YFP cleaved products are detected along with TauRD-YFP in the LMW fractions.

- 6 The corresponding fractions, HMW and LMW species, can be pooled and quantified (using purified TauRD-YFP as standard) by immunoblotting to perform other biochemical assays or cell-based Tau seeding assays.

NOTE: Seeding with 0.5 ng HMW species from TauRD-YFP Clone 10 results in ~20% FRET positive cells while 0.5 ng LMW species results in ~1-2% FRET positive cells following the protocol "Cell-based Tau seeding assay" (Yuste-Checa et al., Nature Communication, 2021).