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Human Brain Sequential Extraction (Tau)

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol details Human Brain Sequential Extraction (Tau). This protocol is an adaptation of the work of several labs.

Attachments



911-2360.pdf

342KB



Materials

Buffers and Reagents

Base Buffer (1L) Store at 4 °C

А	В	С
Final Concentration	Stock Buffer	To Add
10 mM	0.5 M Tris, pH 7.5	20 mL
0.8 M	5 M NaCl	160 mL
1 mM	0.5 M EDTA, pH 7.4	2 mL
10%	Sucrose	100 g
0.1%	Sarkosyl (25%)	4 mL
DI water		To 1 L

25% Sarkosyl (50 mL) Store at \$\mathbb{\mathbb{E}}\$ Room temperature

Note

Sarkosyl powder should be weighed in a fume hood due to its propensity to drift into the lungs.

А	В	
Compound	To Add	
Sarkosyl	12.5 g	
Water	To 50 mL	
Rotate overnight		



0.5 M PMSF (50 mL) Store at 🖁 4 °C

А	В
Compound	To Add
PMSF	4.35 g
100% Ethanol	50 mL

1M DTT (32.5 mL) Aliquot and store at \cdot\ -20 \cdot\ C

А	В	
Compound	To Add	
DTT	1 g	
Water	6.5 mL	
Store in 100 µL aliquots		

Reagents

А	В	С	D
Reagent	Catalog	Vendor	Price
cOmplete Protease Inhibitor	11873580001	Millipore Sigma	\$434
Phosphatase Inhibitor Cocktail 3	P0044-1ML	Millipore Sigma	\$90
Phosphatase Inhibitor Cocktail 2	P5726	Millipore Sigma	\$96
PMSF	P7626-25G	Millipore Sigma	\$311
DDT	D0632-5G	Millipore Sigma	\$140
Sarkosyl	L9150-100G	Millipore Sigma	\$64
27G 1/2 Needle			



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A	В	С	D
19G 1 ½ Needle			

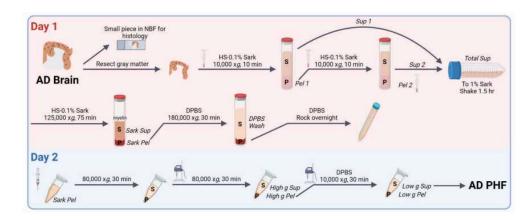
- © COmplete™, EDTA-free Protease Inhibitor Cocktail Merck MilliporeSigma (Sigma-Aldrich) Cotolog #1197050000 Aldrich) Catalog #11873580001
- Phosphatase Inhibitor Cocktail 2 Merck MilliporeSigma (Sigma-Aldrich) Catalog #P5726
- Phosphatase Inhibitor Cocktail 3 Merck MilliporeSigma (Sigma-Aldrich) Catalog #P0044
- **☒** DTT Merck MilliporeSigma (Sigma-Aldrich) Catalog #D0632
- Marck MilliporeSigma (Sigma-Aldrich) Catalog #P7626
- X N-Lauroylsarcosine sodium salt Merck MilliporeSigma (Sigma-Aldrich) Catalog #L9150

Troubleshooting



1 Day Before Extraction

1



Schematic

- 2 Make sure glass 100 or 40 mL homogenizers and pestles (A and B) are cleaned with 70% ethanol, wrapped in foil, and autoclaved.
- 3 Clean out ultracentrifuge tubes and caps with 70% ethanol and dry.
- 4 Transfer brain tissue to \$\selline{-20} \circ freezer the night before (speeds up thaw).
- 5 Ensure there are sufficient protease inhibitor, phosphatase inhibitor, and DTT.

Day 1 - Preparation

- Turn on the ultracentrifuge (**Optima L-100 XP**), set the temperature for 4 °C and turn the vacuum on. Make sure **Ti-45** and **Ti-70** rotors are available. They may be in room 5124.
- 7 Fill 2 buckets with wet ice.
- 8 Warm PMSF to \$\mathbb{8}\$ Room temperature .



- Record information about the tissue to be extracted. Note the weight of the dish to be used for weighing the gray matter.
- Fill a 15 mL conical with 10% neutral buffered formalin (NBF) and label with case number.

Day 1 - Extraction

- 11 Thaw bag(s) of brain tissue § On ice.
- Move brain to petri dish and weigh it.
- Once tissue is thawed, remove meninges and blood vessels. Cut one thin, representative piece
 - off and transfer it to 10% neutral buffered formalin for fixation and subsequent IHC.
- Carefully resect the remaining gray matter from white matter in ice-cold PBS. Transfer gray matter
- Weigh gray matter.
- Prepare 30 mL Extraction Buffer per gram gray matter. Add the following to ice-cold Base

(**1:100**), [M] 0.1 millimolar (mM) PMSF (**1:5000**), [M] 2 millimolar (mM) DDT (**1:500**).

- Add 9 volumes of **Extraction Buffer** to the homogenizer tube. Cut gray matter into small bits,
 - and use a buffer to transfer these bits to the tube.
- Homogenize with **pestle A** until the pestle moves easily.
- Homogenize with **pestle B** 3×25 strokes. Save $200 \, \mu L$ of this as Total Homogenate.



- 20 Pour homogenate evenly into Ti-45 tubes (fits ~ \$\rm 50\text{ mL} \right| /tube). Balance tubes to within 4 0.1 g .
- 21





22 Pour supernatant into 50 mL conical by filtering it through a piece of kimwipe folded into two layers placed in a funnel.

Note Save \triangle 200 μ L of this as Sup 1.

- 23 Add 9 volumes Extraction Buffer to the centrifuge tube and homogenizer. Transfer pellet in buffer to homogenizer.
- 24

Homogenize with **pestle A** until the pestle moves easily.

25 Homogenize with **pestle B** 3× 25 strokes.

> Note Save \triangle 200 μ L of this as Pel 1.

- 26 Pour homogenate evenly into Ti-45 tubes (fits ~50 mL/tube). Balance tubes to within Ä 0.1 g →
- 27 Spin at ₩ 11300 rpm in the **Ti-45** (₩ 10000 x g) for № 00:10:00 at ¥ 4 °C .

10m



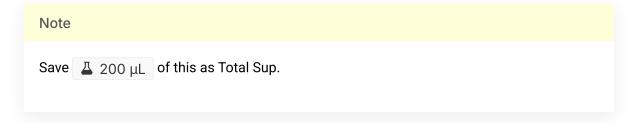
28

- 29 Pour supernatant into 50 mL conical by filtering it through a piece of kimwipe folded into layers placed in a funnel.

During the spin, transfer Sup 1 to a beaker with a stir bar. Add sarkosyl to a final 1%.



30 Combine Sup 1 and Sup 2 in a beaker with a stir bar. Add sarkosyl to a final 1% concentration (1/27).



- 31 Stir *Total Sup* for 1- 01:30:00 at Room temperature 1h 30m
- 32 Add the **Extraction Buffer** to the centrifuge tube and transfer the pellet to the homogenizer. You can use the same vol as Sup 2 for homogenization.

Note • Save \perp 200 μ L equivalent of this as Pel 2. • If you used a smaller volume, correct this by diluting the sample.



33 Add *Total Sup* to Ti-45 tubes. Balance tubes to within $40.1 \,\mathrm{g}$. Spin at

- 1h 15m

- 34 Pipet out supernatant into a beaker. Myelin will have floated. Remove this with a pipette.

Note

- Save \angle 200 μ L of this as Sark Sup.
- At this point, the pellet should be tight, sticky, and red-brown in color.
- 35 Use the vacuum to aspirate remaining liquid around the Sark Pellet.

Note

Do NOT put tubing into centrifuge tube. You can use multiple connected pipet tips, if needed.

36 Add 🗸 10 mL DPBS to each centrifuge tube to wash the Sark Pellet. Use the vacuum to remove DPBS.



37 Add A 2 mL DPBS to wash Sark Pellet a second time. Use vacuum to remove DPBS.

38 Add 🗸 1 mL DPBS to each tube. Pipette liquid towards the Sark Pellet with a cut P1000 tip until the pellet has loosened. Use a transfer pipette to transfer the pellet to a Ti-70 centrifuge



Note

tube.

This step is tricky. Be careful to not lose the Sark Pellet.

39 Use a cut P1000 tip to pipette up and down to resuspend the pellet.



- Add DPBS into the centrifuge tube to reach maximum volume. Balance tubes to within ______ 0.1 g .
- R
- 41 Spin Sark Pel at \$\infty\$ 50000 rpm in the **Ti-70** (\$\infty\$ 18000 x g) for \$\infty\$ 00:30:00 at \$\infty\$ 4 °C .
- 30m
- 42 Save $\[\]$ 200 μ L of the supernatant as DPBS wash. Remove the remaining supernatant by vacuum.
- 1 Page
- Add \perp 100 μ L DPBS/**1 g** tissue to the pellet. Use a cut P1000 tip to first loosen the pellet, then break it up as much as possible without pipetting up and down. Transfer to 1.5 mL tubes.

Note

This is a tricky step as the pellet may stick to the pipette tip.

- Vortex briefly and spin in a tabletop centrifuge at 1000 x g for 00:01:00.
- 1m

Rock the tube at 4 °C Overnight .

7

Day 1 - Cleanup

- Bleach all homogenizers, tools and tubes, but use only soap for metal lids.
- A7 Rinse centrifuge tubes for 500:10:00 after bleaching.



48 Bleach out ice bucks.



49 Bleach vacuum flask and rinse out.



Day 2

- 50 Move fixed brain to leaching buffer and cassette for paraffin embedding.
- 51 Spin in a tabletop centrifuge at **(4)** 1000 x g for **(5)** 00:01:00 at **(4)** 4 °C.

1m

52 Pass the suspension repeatedly through a 27G ½ needle to homogenize.

Note

If the clumps are large, start with the bigger 19G 1 ½ needle. Centrifuge at 1000 x g for 00:01:00 if needed to bring materials to the bottom of the tube.

- 53 Transfer the resuspended Sark Pel to an autoclaved 1.5 mL Beckman ultracentrifuge tube.
- 54 Add 4 100 µL DPBS to the centrifuge tube and resuspend well.



Note

Save \perp 30 μ L as Sark Pel.

55 Balance tubes to within 0.1g. Spin in OptimaMAX-TL (room 5124) (TLA100.3 rotor with adaptors) at \$\mathbb{3} 45000 \text{ rpm} (\mathbb{8} 80000 \text{ x g}) for \mathbb{\infty} 00:30:00 at \$\mathbb{8} 4 \cdot \cdo



56 Remove supernatant and add \perp 100 μ L DPBS/1 g tissue to the pellet.





Vortex to mix. Freeze or continue to process Sark Pellet.



Day 2 or 3 - Further Purification



- 58 Thaw the Sark Pellet.
- 59 Sonicate the Sark Pellet with 20×1 sec pulses with hand sonicator.
- Balance tubes to within 0.1g. Spin in **OptimaMAX-TL** (room 5124) (**TLA100.3** rotor with adaptors) at 45000 rpm (80000 x g) for 00:30:00 at 4 °C.



- Transfer supernatant into another tube as High g Sup using sterile pipette tips.
- Resuspend the pellet with 20% of the volume of DPBS (e.g. for Δ 700 μ L Sup, add Δ 140 μ L).



Sonicate High g Pellet for 90-120x 00:00:01 pulses with hand sonicator on ice to break up the pellet.



Note

Save $\stackrel{\perp}{\bot}$ 10 μL as High g Pel.

Transfer the resuspended High g Pellet to a 1.5 mL tube. Spin in tabletop centrifuge at 10000 x g for 00:30:00 at 4 oc .



Transfer supernatant using a sterile pipette tip to a 1.5 mL tube.



Note

- Save Δ 20 μL as Low g Sup.
- This is the final supernatant that CONTAINS ENRICHED PATHOLOGICAL TAU.

66 Add an **equal volume** of DPBS to the pellet and sonicate 30x 00:00:01 pulses with hand sonicator to resuspend the pellet.





Note

Save $\underline{\underline{}}$ 200 $\mu \underline{L}$ of the pellet as Log g Pel.