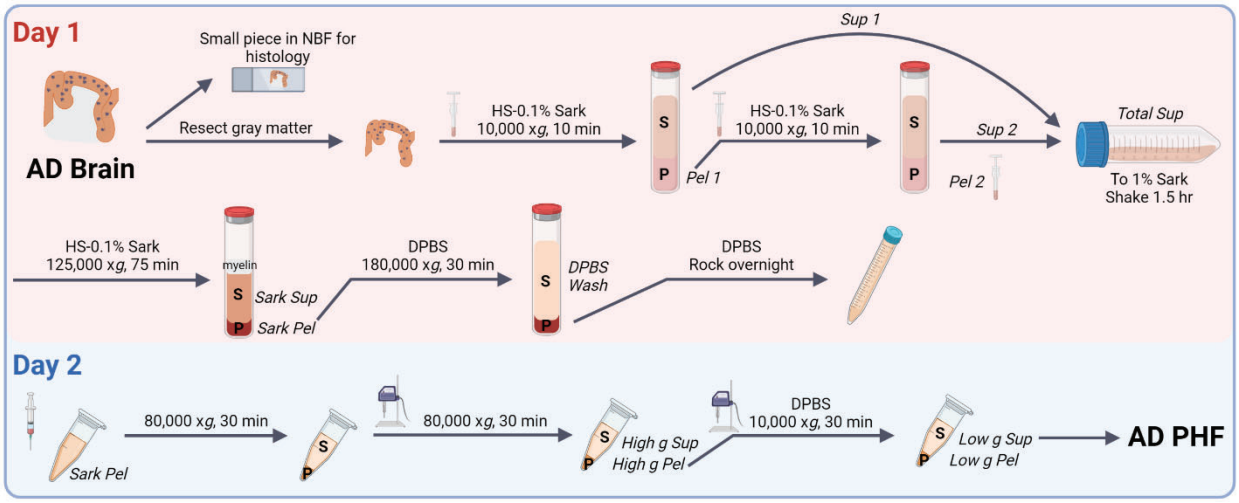
Human Brain Sequential Extraction (Tau) MXH_06.27.2022

Schematic



1 Day Before Extraction

1. Sign up for the ultracentrifuge (Optima L-100 XP).
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 -20°C freezer the night before (speeds up thaw).
5. Ensure there are sufficient protease inhibitor, phosphatase inhibitor, and DTT.

Day 1

Preparation

1. 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.
2. Fill 2 buckets with wet ice.
3. Warm PMSF to room temperature.
4. Record information about the tissue to be extracted. Note the weight of the dish to be used for weighing gray matter.
5. Fill a 15 mL conical with 10% neutral buffered formalin (NBF) and label with case number.

Extraction

1. Thaw bag(s) of brain tissue on wet ice.
2. Move brain to petri dish and weigh it.
3. 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.
4. Carefully resect remaining gray matter from white matter in ice-cold PBS. Transfer gray matter to a petri dish on ice to be weighed.
5. Weigh gray matter.

6. Prepare **30 mL Extraction Buffer** per gram gray matter. Add the following to ice-cold **Base Buffer**: cOmplete protease inhibitor (**1 tab/50 mL**), phosphatase inhibitors (2&3) (**1:100**), 0.1 mM PMSF (**1:5000**), 2 mM DDT (**1:500**).
7. Add 9 volumes of **Extraction Buffer** to the homogenizer tube. Cut gray matter into small bits, and use buffer to transfer these bits to the tube.
8. Homogenize with **pestle A** until the pestle moves easily.
9. Homogenize with **pestle B** *3x 25* strokes. Save **200 µL** of this as *Total Homogenate*.
10. Pour homogenate evenly into Ti-45 tubes (fits ~50 mL/tube). Balance tubes to within 0.1g.
11. Spin at **11,300 rpm** in the **Ti-45** (10,000 x*g*) for **10 min** at **4°C**.
12. Pour supernatant into 50 mL conical by filtering it through a piece of kimwipe folded into two layers placed in a funnel. Save **200 µL** of this as *Sup 1*.
13. Add 9 volumes **Extraction Buffer** to the centrifuge tube and homogenizer. Transfer pellet in buffer to homogenizer.
14. Homogenize with **pestle A** until the pestle moves easily.
15. Homogenize with **pestle B** *3x 25* strokes. Save **200 µL** of this as *Pel 1*.
16. Pour homogenate evenly into Ti-45 tubes (fits ~50 mL/tube). Balance tubes to within 0.1g.
17. Spin at **11,300 rpm** in the **Ti-45** (10,000 x*g*) for **10 min** at **4°C**.
18. During the spin, transfer *Sup 1* to a beaker with a stir bar. Add sarkosyl to a final 1%.
19. Pour supernatant into 50 mL conical by filtering it through a piece of kimwipe folded into two layers placed in a funnel. Save **200 µL** of this as *Sup 2*.
20. Combine *Sup 1* and *Sup 2* in a beaker with a stir bar. Add sarkosyl to a final 1% concentration (**1/27**). Save **200 µL** of this as *Total Sup*.
21. Stir *Total Sup* for 1-1.5 hours at RT.
22. Add **Extraction Buffer** to the centrifuge tube and transfer pellet to homogenizer. You can use the same vol as *Sup 2* for homogenization. Save **200 µL equivalent** of this as *Pel 2*. If you used a smaller volume, correct for this by diluting the sample.
23. Add *Total Sup* to Ti-45 tubes. Balance tubes to within 0.1g. Spin at **40,000 rpm** in the **Ti-45** (125,000 x*g*) for **75 min** at **4°C**.
24. Pipet out supernatant into a beaker. Myelin will have floated. Remove this with a pipette. Save **200 µL** of this as *Sark Sup*.
    * *At this point, the pellet should be tight, sticky, and red-brown in color.*
25. Use the vacuum to aspirate remaining liquid around the *Sark Pellet*.
    **Do NOT put tubing into centrifuge tube. You can use multiple connected pipet tips, if needed.*
26. Add **10 mL** DPBS to each centrifuge tube to wash the *Sark Pellet*. Use vacuum to remove DPBS.
27. Add **2 mL** DPBS to wash *Sark Pellet* a second time. Use vacuum to remove DPBS.
28. 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 tube.
    *This step is tricky. Be careful to not lose the *Sark Pellet*.
29. Use a cut P1000 tip to pipette up and down to resuspend the pellet.
30. Add DPBS into the centrifuge tube to reach maximum volume. Balance tubes to within 0.1g.
31. Spin *Sark Pel* at **50,000 rpm** in the **Ti-70** (180,000 x*g*) for **30 min** at **4°C**.
32. Save **200 µL** of the supernatant as *DPBS wash*. Remove remaining supernatant by vacuum.

33. Add **100 µL** 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.
    **This is a tricky step as the pellet may stick to the pipette tip*.
34. Vortex briefly and spin in a tabletop centrifuge at **1000 x*g*** for **1 minute**.
35. Rock the tube at **4°C** overnight.

Cleanup

1. Bleach all homogenizers, tools and tubes, but use only soap for metal lids.
2. Rinse centrifuge tubes for 10 minutes after bleaching.
3. Bleach out ice bucks.
4. Bleach vacuum flask and rinse out.

Day 2

1. Move fixed brain to leaching buffer and cassette for paraffin embedding.
2. Spin in a tabletop centrifuge at **1000 x*g*** for **1 minute** at **4°C**.
3. Pass the suspension repeatedly through a 27G ½ needle to homogenize.
   If the clumps are large, start with the bigger 19G 1 ½ needle. Centrifuge at **1000 xg for **1 minute** if needed to bring materials to the bottom of the tube.*
4. Transfer the resuspended *Sark Pel* to an autoclaved 1.5 mL Beckman ultracentrifuge tube.
5. Add **100 µL** DPBS to the centrifuge tube and resuspend well. Save **30 µL** as *Sark Pel*.
6. Balance tubes to within 0.1g. Spin in **OptimaMAX-TL** (room 5124) (**TLA100.3** rotor with adaptors) at **45,000 rpm** (80,000 x*g*) for **30 minutes** at **4°C**.
7. Remove supernatant and add **100 µL** DPBS/**1 g** tissue to the pellet.
8. Vortex to mix. Freeze or continue to process *Sark Pellet*.

Day 2 or 3 – Further Purification

1. Thaw the *Sark Pellet*.
2. Sonicate the *Sark Pellet* with 20x 1 sec pulses with hand sonicator.
3. Balance tubes to within 0.1g. Spin in **OptimaMAX-TL** (room 5124) (**TLA100.3** rotor with adaptors) at **45,000 rpm** (80,000 x*g*) for **30 minutes** at **4°C**.
4. Transfer supernatant into another tube as *High g Sup* using sterile pipette tips.
5. Resuspend the pellet with **20%** of the volume of *DPBS* (e.g. for 700 µL *Sup*, add 140 µL).
6. Sonicate *High g Pellet* for **90-120x 1 sec** pulses with hand sonicator on ice to break up the pellet. Save **10 µL** as *High g Pel*.
7. Transfer the resuspended *High g Pellet* to a 1.5 mL tube. Spin in tabletop centrifuge at **10,000 x*g*** for **30 minutes** at **4°C**.
8. Transfer supernatant using a sterile pipette tip to a 1.5 mL tube. Save **20 µL** as *Low g Sup*.
   This is the final supernatant that CONTAINS ENRICHED PATHOLOGICAL TAU.
9. Add an **equal volume** of DPBS to the pellet and sonicate **30x 1 sec** pulses with hand sonicator to resuspend the pellet. Save **200 µL** of the pellet as *Log g Pel*.

Buffers and Reagents

Base Buffer (1L) *Store at 4°C*

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 RT.*

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

12.5 g Sarkosyl. Fill to 50 mL with water. Rotate overnight.

0.5 M PMSF (50 mL) *Store at 4°C.*

4.35 g PMSF in 50 mL 100% Ethanol

1 M DTT (32.5 mL) *Aliquot and store at -20°C.*

Dissolve 1 g DTT in 6.5 mL water. Store in 100 µL aliquots.

Reagents

Reagent	Catalog	Vendor	Price
cOmplete Protease Inhibitor	11873580001	Millipore Sigma	$434
Phosphatase Inhibitor Cocktail 3	P0044-1ML	Millipore Sigma	$90
Phosphatase Inhibitor Cocktail 2	P5726-1ML	Millipore Sigma	$96
PMSF	P7626-25G	Millipore Sigma	$311
DDT	D0632-5G	Millipore Sigma	$140
Sarkosyl	L9150-100G	Millipore Sigma	$64
27G ½ Needle			
19G 1 ½ Needle			