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Protocol status: Working
We use this protocol and it's working

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Human Brain Sequential Extraction (Tau)

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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

MATERIALS

Buffers and Reagents

Base Buffer (1L) Store at 4 °C

	A	B	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 Room temperature

Note

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

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91707

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	A	B
	Compound	To Add
	Sarkosyl	12.5 g
	Water	To 50 mL
	Rotate overnight	

0.5 M PMSF (50 mL) Store at 4 °C

	A	B
	Compound	To Add
	PMSF	4.35 g
	100% Ethanol	50 mL

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

	A	B
	Compound	To Add
	DTT	1 g
	Water	6.5 mL
	Store in 100 µL aliquots	

Reagents

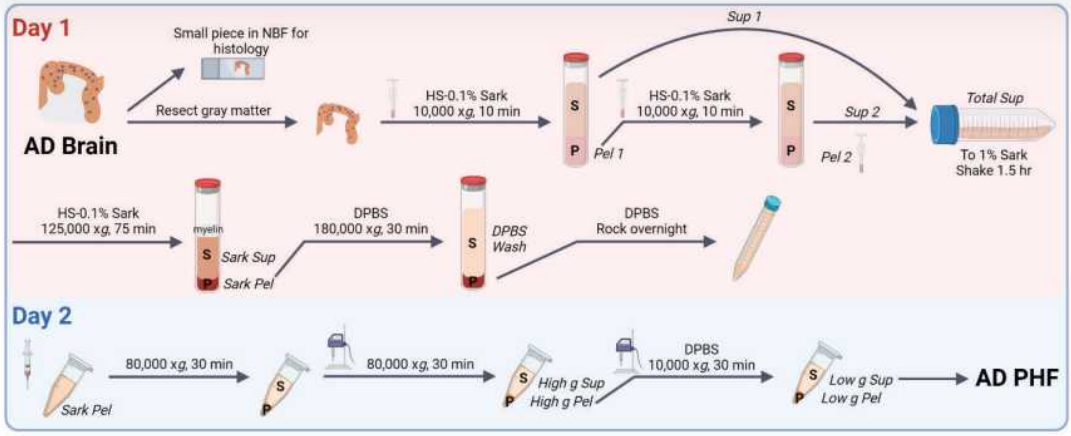
	A	B	C	D
	Reagent	Catalog	Vendor	Price
	cOmplete Protease Inhibitor	11873580001	Millipore Sigma	$434
	Phosphatase Inhibitor Cocktail 3	P0044-1ML	Millipore Sigma	$90

	A	B	C	D
	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 ½ Needle			
	19G 1 ½ Needle			

- cOmplete™, EDTA-free Protease Inhibitor Cocktail **Merck MilliporeSigma (Sigma-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**
- PMSF **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P7626**
- N-Lauroylsarcosine sodium salt **Merck MilliporeSigma (Sigma-Aldrich) Catalog #L9150**

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 -20 °C freezer the night before (speeds up thaw).

5 Ensure there are sufficient protease inhibitor, phosphatase inhibitor, and DTT.

Day 1 - Preparation

6 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 Room temperature.

9 Record information about the tissue to be extracted. Note the weight of the dish to be used for weighing the gray matter.

10 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 .

12 Move brain to petri dish and weigh it.

13 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.

14 Carefully resect the remaining gray matter from white matter in ice-cold PBS. Transfer gray matter to a petri dish On ice to be weighed.

15 Weigh gray matter.

16 Prepare 30 mL **Extraction Buffer** per gram gray matter. Add the following to ice-cold B**ase Buffer**: cOmplete protease inhibitor (**1 tab**/ 50 mL), phosphatase inhibitors (2&3) (**1:100**), [M] 0.1 millimolar (mM) PMSF (**1:5000**), [M] 2 millimolar (mM) DDT (**1:500**).

17 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.

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

19 Homogenize with **pestle B** 3x 25 strokes. Save 200 µL of this as Total Homogenate.

20 Pour homogenate evenly into Ti-45 tubes (fits ~ 50 mL /tube). Balance tubes to within 0.1 g.

21 Spin at 11300 rpm in the **Ti-45** (10000 x g) for 00:10:00 at 4 °C. 10m

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 200 µL 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** 3x 25 strokes.

> **Note**
>
> Save 200 µL of this as Pel 1.

26 Pour homogenate evenly into Ti-45 tubes (fits ~50 mL/tube). Balance tubes to within 0.1 g.

27 Spin at 11300 rpm in the **Ti-45** (10000 x g) for 00:10:00 at 4 °C. 10m

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

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

> **Note**
>
> Save 200 µL of this as Sup 2.

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

Note

Save 200 µL of this as Total Sup.

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 200 µL 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 0.1 g. Spin at 40000 rpm in the Ti- 1h 15m
125000 x g) for 01:15:00 at 4 °C.

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

Note

- Save 200 µL 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 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 tube.

Note

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.

40 Add DPBS into the centrifuge tube to reach maximum volume. Balance tubes to within 0.1 g.

41 Spin Sark Pel at 50000 rpm in the **Ti-70** (18000 x g) for 00:30:00 at 4 °C . 30m

42 Save 200 µL of the supernatant as DPBS wash. Remove the remaining supernatant by vacuum.

43 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.

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

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

45 Rock the tube at 4 °C Overnight.

Day 1 - Cleanup

46 Bleach all homogenizers, tools and tubes, but use only soap for metal lids.

47 Rinse centrifuge tubes for 00:10:00 after bleaching. 10m

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 1000 x g for 00:01:00 at 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 100 µL DPBS to the centrifuge tube and resuspend well.

Note

Save 30 µL as Sark Pel.

55 Balance tubes to within 0.1g. Spin in **OptimaMAX-TL** (room 5124) (**TLA100.3** rotor with adaptors) at 30m 45000 rpm (80000 x g) for 00:30:00 at 4 °C .

56 Remove supernatant and add 100 µL DPBS/1 g tissue to the pellet.

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

Day 2 or 3 – Further Purification

1h 0m 2s

58 Thaw the Sark Pellet.

59 Sonicate the Sark Pellet with 20x 1 sec pulses with hand sonicator.

60 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 . 30m

61 Transfer supernatant into another tube as High g Sup using sterile pipette tips.

62 Resuspend the pellet with **20%** of the volume of DPBS (e.g. for 700 µL Sup, add 140 µL).

63 Sonicate High g Pellet for 90-120x 00:00:01 pulses with hand sonicator On ice to break up t 1s pellet.

Note

Save 10 µL as High g Pel.

64 Transfer the resuspended High g Pellet to a 1.5 mL tube. Spin in tabletop centrifuge at 10000 x g 30m 00:30:00 at 4 °C.

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

Note

- Save 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 sonicato 1s to resuspend the pellet.

Note

Save 200 µL of the pellet as Log g Pel.