Single soma isolation

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 We use this protocol and it's working

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Isolation of single somas from postmortem fresh frozen human brain and immunostaining for AT8 and MAP2

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ABSTRACT

- Protocol optimized to isolate single neuronal somas with cytoplasmic protein aggregates from postmortem fresh frozen brain
- Works for the isolation of neuronal somas with neurofibrillary tangles from Alzheimer disease's brains
- Note that cell membranes are highly disrupted in tissues that were previously frozen. Thus, cytoplasmic transcripts are largely lost. This protocol is therefore useful for the isolation of specific cell populations using antibodies against cytoplasmic antigens, but not for the profiling of cytoplasmic mRNA.

ATTACHMENTS

[Single soma isolation from postmortem frozen human brain_CobosLab_10282019.pdf](#)

MATERIALS

MATERIALS

- DNase I (RNase-free) - 1,000 units New England Biolabs Catalog #M0303S
- Bovine Serum Albumin (BSA) Sigma Aldrich Catalog #A7906
- Magnesium chloride hexahydrate Sigma Aldrich Catalog #M2670
- UltraPure™ DNase/RNase-Free Distilled Water Thermo Fisher Scientific Catalog #10977023
- Sucrose Fisher Scientific Catalog #S25590B

Keywords: human brain tissue, postmortem, immunostaining, AT8, MAP2, FACS, single neuronal somas, isolation, tau tangles, NFT

	Potassium chloride solution Millipore Sigma Catalog #60142
	UltraPure™ 1M Tris-HCl pH 8.0 Thermo Fisher Scientific Catalog #15568025
	DL-Dithiothreitol Sigma Aldrich Catalog #D9779-1G
	cOmplete™ Protease Inhibitor Cocktail Sigma Aldrich Catalog #11697498001
	NxGen® RNase Inhibitor Lucigen Catalog #30281-2
	Anti-Microtubule-Associated Protein 2 (MAP2) Antibody Merck Millipore Catalog #AB5622
	Phospho-Tau (Ser202 Thr205) Monoclonal Antibody (AT8) Thermo Fisher Scientific Catalog #MN1020
	SYTOX™ Green Nucleic Acid Stain Thermo Fisher Scientific Catalog #S7020
	Goat anti-Rabbit IgG (H L) Alexa Fluor 647 Thermo Fisher Scientific Catalog #A-21245
	Goat anti-Mouse IgG (H L) Alexa Fluor 350 Thermo Fisher Scientific Catalog #A-11045
	OptiPrep™ Density Gradient Medium Sigma Aldrich Catalog #D1556

- Material and tools needed: Forceps, spatula, blades, dounce tissue grinder (Potter-Elvehjem tissue grinder), cell strainers, petri dishes

Equipment	
Potter-Elvehjem Tissue Grinders	NAME
Tissue Grinder	TYPE
DWK Life Sciences	BRAND
8860000022	SKU
https://www.fishersci.com/shop/products/kimble-chase-kontes-potter-elvehjem-tissue-grinders-ptfe-pestle-unground-glass-tub-size-22/k8860000022	LINK

Buffer preparation (All solutions should be RNase-free for single-soma RNAseq experiments):

Isolation Medium #1 (IM1), 45 ml (optional)

Prepare in a 50 ml Falcon tube and store at 4 °C up to 6 months.

Amount	Reagent	Final concentration
7500 µl	1.5 M Sucrose	250 mM
1125 µl	1 M KCl	25 mM
225 µl	1 M MgCl ₂	5 mM
450 µl	1 M Tris (pH 8)	10 mM
35.7 ml	H ₂ O	

Homogenization Buffer (3 ml per sample)

Prepare FRESH and keep ICED or at 4 °C. Discard after use.

Amount	Reagent	Final concentration
2955 µl	IM1	
3 µl	DTT 1mM	1 µM
30 µl	50x Protease Inhibitor	0.5 x
15 µl	RNaseIN 40 U/µl	0.2 U/µl
No Triton !		

Iodixanol dilutions

Prepare in a 50 ml Falcon tubes and store at 4 °C up to 6 months. Accuracy with Iodixanol and sucrose concentrations is critical.

■ Iodixanol medium (IDM), 45 ml

Amount	Reagent	Final concentration
7500 μ l	1.5 M Sucrose	250 mM
3750 μ l	1 M KCl	83 mM
750 μ l	1 M $MgCl_2$	17 mM
1500 μ l	1 M Tris (pH 8)	33 mM
31.5 ml	H ₂ O	

■ **Iodixanol 42 %(v/v), 20 ml**

14 ml Iodixanol 60 %(v/v) + 6 ml IDM

■ **Iodixanol 25 %(v/v), 30 ml**

12.5 ml Iodixanol 60 %(v/v) + 17.5 ml IDM

Freezing Storage Buffer (FSB), 15ml (optional)

Prepare in a 50 ml Falcon tube and store at 4 °C up to 6 months.

Amount	Reagent	Final concentration
1.665 ml	1.5 M Sucrose	166.5 mM
75 μ l	1 M $MgCl_2$	5 mM
150 μ l	1 M Tris (pH8)	10 mM
13.1 ml	H ₂ O	

Buffer for Immunostaining, 10ml

Amount	Reagent	Final concentration

	9400 μ l	RNase-free PBS (pH 7.4)	
	500 μ l	RNase-free 10 % BSA	0.5 %
	50 μ l	1 M MgCl ₂	5 mM
	10 μ l	DNase I (2000 U/ml)	2 U/ml

Buffer for Antibody Incubation

Add to 1 ml of Buffer for Immunostaining:

Amount	Reagent	Final concentration
5 μ l	RNaseIN 40 U/ μ l	0.2 U/ μ l

Collection medium for FACS (1 ml; 0.2 ml/vial)

Amount	Reagent	Final concentration
950 μ l	RNase-free PBS (pH 7.4)	
25 μ l	RNaseIN	
*	BSA 10% *After collecting!	1 %

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

Uses fresh human brain tissue - Biosafety Level 2 lab work.

BEFORE START INSTRUCTIONS

- Aims for **minimal mechanical damage** (as little centrifugation and pipetting as possible)
- All solutions and materials should be **RNase free** and **kept iced or at** **4 °C** at all times
- Glassware and metal tools are **sealed with aluminum foil** and **baked at** **220 °C** **for** **06:00:00**

1 Prepare **Homogenization Buffer** and cool it on ice.

Amount	Reagent	Final concentration
2955 µl	IM1	
3 µl	DTT 1mM	1 µM
30 µl	50x Protease Inhibitor	0.5 x
15 µl	RNaseIN 40 U/µl	0.2 U/µl
No Triton!		

Homogenization Buffer (3 ml per sample)

Prepare FRESH and keep ICED or at **4 °C** . Discard after use.

1.1 Add 2955 μL IM1 .

1.2 Add 3 μL DTT
1mM .

1.3 Add 30 μL 50x Protease
Inhibitor .

1.4 Add 15 μL RNaseIN 40
U/ μl .

2 Pre-cool the dounce tissue grinder on ice (**Potter-Elvehjem tissue grinder**, 8 mL tubes, 0.1–0.15 mm clearance space between pestle and tubes).

3 Add 2.4 mL of **Homogenization buffer** to the dounce tissue grinder.

4 Collect the brain chunk (~ 200 mg) and transfer to a Petri dish on ice.

5 Cut out into small pieces using a chilled scalpel or blade.

6 Transfer all pieces into the dounce tissue grinder.

- 7 Slowly homogenize the tissue, on ice (**8~15 strokes; slow**).
Check on **hematocytometer** while homogenizing and adjust the number of strokes.

Note

Too few strokes – you will see clumps; too many strokes – you will get a higher proportion of naked nuclei.

- 8 Filter homogenate using a **100 µm** Corning cell strainer to remove clumps.
--> Take sample for hemocytometer.

- 9 Transfer the homogenate into two precooled 1.5 ml Eppendorf tubes.

10

Centrifuge at 400 x g for 00:05:00 at 4 °C .

11

Slowly aspirate the supernatant from the side of the tube.
Avoid disturbing the pellets, up to 50 µL of supernatant can be left.

12

Gently **resuspend** each pellet in 225 µL (final volume) of cold **Homogenization buffer** and pool both tubes (final volume 450 µL).

13

Add an **equal** volume of cold **42% v/v iodixanol**.

Note

Critical! Be very precise with volumes. 450 µL suspension + 450 µL 50% iodixanol. Final iodixanol concentration is **21%**.

14 Gently pipette mix.

15 Add an equal volume of **25% iodixanol** (900 µL) into a 2 ml Eppendorf tube.

16 Slowly layer off the 21% iodixanol suspension mix over the 25% iodixanol, without mixing them.

17 Centrifuge at 8000 x g for 00:15:00 at 4 °C .

18 Prepare **buffer for Immunostaining**.

Amount	Reagent	Final concentration
9400 µl	RNase-free PBS (pH7.4)	
500 µl	RNase-free 10 % BSA	0.5 %
50 µl	1 M MgCl ₂	5 mM
10 µl	DNAse I (2000 U/ml)	2 U/ml

18.1 Add 9400 µL RNase-free PBS (pH7.4)

18.2 Add 500 µL RNase-free 10 % BSA

18.3 Add 50 µL 1 M MgCl₂

18.4 Add 10 µL DNase I (2000 U/ml)

19 Prepare **buffer for Antibody incubation** (1.5 ml per sample) by adding 5 µL of RNaseIN 40 U/µl to 1 mL of **buffer for Immunostaining** (final concentration 0.2U/µl)

20 After centrifugation, remove and discard the top myelin-rich debris layer (you can use a 1 ml pipette with the tip cut, or cotton swabs).

21 Remove and discard the aqueous supernatant, without disrupting the **somas pellet**. Avoid contaminating with the top layer.

22 Use a small amount of **buffer for Antibody Incubation** to resuspend the pellet and transfer the solution to a new tube.

23 Gently resuspend in 200 μL of **buffer for Antibody Incubation**.

24 Incubate for 00:15:00 , at 4 °C or iced, for **blocking** nonspecific staining.

25 Take sample for hemocytometer.

26 Take a sample for **unstained** control. Take sample for **2AB**-only (nonspecific binding control).

27 Add primary antibodies (**Ms-a-AT8 1:150** and **Rb-a-MAP2 1:40**) and incubate on a shaker in cold room (4 °C) for 00:40:00 .

Note

Use Eppendorfs coated with BSA for collection (to coat the Eppendorf tubes, fill them with 10% BSA solution in PBS for 00:05:00 , rinse with PBS, and dry at 4 °C overnight).

Washing

28 Add 500 μL of **buffer for Immunostaining** and invert several times.

29 Centrifuge at 400 x g for 00:05:00 at 4 °C .

30 Carefully transfer all the supernatant (don't leave any supernatant! It's ok to drag some pellet) to a new Eppendorf tube and resuspend the pellet in 400 µL of **buffer for Antibody Incubation**.

31 Centrifuge the supernatant at 500 x g for 00:05:00 at 4 °C to recover non-pelleted somas.

32 Resuspend them in 200 µL of **buffer for Antibody Incubation** and pool for a final volume of 600 µl.

33 Take samples for single color staining.

34 Add secondary antibodies (**G-a-Ms Alexa350, 1:500** and **G-a-Rb Alexa647, 1:500**) and **Sytox Green 1:400** (1:100 stock, final concentration **1:40.000**)

35 Check the quality of the sample on hemocytometer.

Note

For 200 mg of tissue, we should have ~ 1-2 Million somas in ~0.6 mL.

36 Transfer to 7 ml culture tubes.

37 Place on ice and bring them to the FACS facility for sorting.

Note

FACS sorting controls:

Non-stained

2AB only

Single stainings

- 38** Collect in BSA-coated Eppendorfs containing **collection medium** (volume of collection medium should be ~1/5 of the expected final volume after collection).

- 39** Add BSA after collection for 1 % BSA final concentration (tested for 10x Chromium v2 and v3).

Note

Different Single Cell assays tolerate different BSA concentrations, but lowering it may increase nuclei aggregation.