Jan 13, 2026

Nuclei isolation of fresh frozen human brain samples for for scRNA-seq

DOI
https://dx.doi.org/10.17504/protocols.io.5qpvo1rddg4o/v1

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Protocol Citation: Koen Theunis, Suresh Poovathingal, Sarah Geurs 2026. Nuclei isolation of fresh frozen human brain samples for for scRNA-seq. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.5qpvo1rddg4o/v1**

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

Created: September 09, 2025

Last Modified: January 13, 2026

Protocol Integer ID: 238288

Keywords: ASAPCRN, scRNA-seq, nuclei isolation, nuclei isolation for scrna, nuclei isolation of fresh frozen human brain sample, fresh frozen human brain sample, scrna, nuclei isolation, seq, nuclei

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-000430
Aligning Science Across Parkinson's
Grant ID: ASAP-025179

Abstract

This protocol describes nuclei isolation for scRNA-seq.

Attachments

XLSX

SN-Isolation-forRNA_...

18KB

Materials

	A	B
	HOW MANY SAMPLES TO PROCESS?	**2**
	Concentration of Sucrose?	**2 M**

2X salt-Tris solution

	A	B	C	D
	2X salt-Tris solution	**Stock conc**	**Final conc**	**V (µl)**
	NaCl	5000 mM	292 mM	1168.0
	Tris pH7.5	1000 mM	20 mM	400.0
	CaCl	1000 mM	2 mM	40.0
	MgCl2	1000 mM	42 mM	840.0
	Water			17552.0
	Total Volume			20000.0

TW20+ Homogenization buffer

	A	B	C	D
	TW20+ Homogenization buffer	**Stock conc**	**Final conc**	**V (µl)**
	2X salt-Tris solution	2 X	1 X	1100.0
	Tween-20	10%	0.03%	6.6
	BSA	7.5%	0.01%	2.9
	KCl	3000 mM	25 mM	18.3
	Sucrose	2000 mM	250 mM	275.0
	DTT*	1000 mM	1 mM	2.2
	cOmplete protease inhibitor (Roche EDTA free)*	50 X	0.5 X	22.0
	RNasin Plus (Promega)*	40 U/ul	0.5 U/ul	27.5
	Water			745.4

	A	B	C	D
	Total Volume			**2200.0**

TW20- Homogenization buffer

	A	B	C	D
	TW20- Homogenization buffer	**Stock conc**	**Final conc**	**V (µl)**
	2X salt-Tris solution	2 X	1 X	572.0
	BSA	7.5%	0.01%	1.5
	KCl	3000 mM	25 mM	9.5
	Sucrose	2000 mM	250 mM	143.0
	DTT*	1000 mM	1 mM	1.1
	cOmplete protease inhibitor (Roche EDTA free)*	50 X	0.2 X	4.6
	RNasin Plus (Promega)*	40 U/ul	0.2 U/ul	5.7
	Water			406.5
	Total Volume			**1144.0**

GM (Gradient Medium)

	A	B	C	D
	GM (Gradient Medium)	**Stock conc**	**Final conc**	**V(µl)**
	CaCl	1000 mM	1 mM	1.1
	Optiprep*	60%	50%	953.3
	MgCl2	1000 mM	5 mM	5.7
	Tris pH 7.5	1000 mM	10 mM	11.4
	Sucrose	2000 mM	75 mM	42.9
	DTT*	1000 mM	1 mM	1.1
	cOmplete protease inhibitor*	50 X	0.5 X	11.4
	RNasin Plus (Promega)*	40 U/ul	0.5 U/ul	14.3
	Water			102.6

	A	B	C	D
	Total Volume			**1144**

ODM (Optiprep Diluent Medium)

	A	B	C	D
	ODM (Optiprep Diluent Medium)	**Stock conc**	**Final conc**	**V(µl)**
	KCl	3000 mM	150 mM	48.1
	MgCl2	1000 mM	30 mM	28.9
	Tris pH 8	1000 mM	60 mM	57.8
	Sucrose	2000 mM	250 mM	120.3
	Water			707.6
	Total Volume			**963**

29% Cushion

	A	B	C	D
	29% Cushion	**Stock conc**	**Final conc**	**V(µl)**
	Optiprep	60%	29%	819
	ODM			875
	Total Volume			**1694**

Resuspension Buffer

	A	B	C	D
	Resuspension Buffer	**Stock conc**	**Final conc**	**V(µl)**
	PBS	1 X	1 X	192.5
	BSA	7.5%	0.75%	22
	RNasin Plus (Promega)*	40 U/uL	1 U/uL	5.5
				220

*Add these components just before use.

A. Preparation

5m

1 Prepare the 2X salt-Tris solution and aliquot to 1 ml tube and freeze at -20 °C .

2 Put the centrifuge for 4 °C .

3 Prepare the tween-20 lysis buffer fresh for each preparations.

4 Prepare cOmplete protease inhibitor (50X) by dissolving a tablet in 1 mL of water.

5 Filter sucrose solution and Protease inhibitor through 0.2 um/0.45 um filter.

6 Thaw all the reagents and prepare for the buffer reagents.

7 Place the homogenizer at -70 °C at the start of the experiment and place On ice 00:05:00 before homogenization.

5m

B. Homogenization

8m

8 Cut brain piece on dry ice (if large; > 20 mg - 50 mg tissue).

9 Transfer 750 µL **of ice-cold TW20+ HBuffer** and tissue pieces to a homogenizer and leave it in +HB for 00:03:00 before starting homogenization.

3m

10 Homogenize tissue with **10** gentle strokes with **pestle A**, and **10** gentle strokes with **pestle B**.

11 Put homogenate through 70 µm cell strainer, wash homogenizer and filter with 250 µL **TW+HB** and leave the filterate On ice . **Start the timer for 5 mins.**

12 Transfer the filterate to a 2 mL protein low bind eppendorf and centrifuge @ 400 x g - 500 x g for 00:05:00 . Discard the supernatant. 5m

13 Resuspend the pellet gently in 200 µL **of TW20- HB**, and transfer to 2 mL low-bind tube.

14 Add additional **TW20- HB** to reach a final volume of 520 µL .

15 Add 520 µL of Gradient Medium (Vf = 1040 µL).

C. Isolation by centrifugation 20m

16 Layer 770 µL of 29% Cushion in the ultracentrifuge tube.

17 Layer the sample on top of cushion using a P1000, without disturbing the cushion.

18 Check tubes weight, adjust weight with HB/GM.

19 Centrifuge at least 3000 rcf with a swinging bucket rotor, at 4 °C for 00:20:00 with brake off. 20m

20 Remove supernatant, remove the lower supernatant with P200 or less, leaving around 50 µL - 100 µL .

D. Resuspension and counting 5m

21 Disrupt nuclei on bottom by pipetting with P200. And bring total volume to a fresh 1.5 mL LoBind tube.

22 Add 100 µL of 1X Resuspension buffer to ultracentrifuge tube to retain extra nuclei, and add them to the 1.5 mL tube.

23 Spin 00:05:00 @ 350 x g - 450 x g followed by resuspension in Resuspension buffer. Optional filter and count. 5m

Protocol references

This protocol was based on earlier work from others but with in-house modifications:

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