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Nuclei isolation from human brain cortex

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Xian Adiconis[1], Joshua Z Levin[1]

[1]Broad Institute of MIT and Harvard

eQTL

xian Adiconis
Broad Institute

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We use this protocol and it's working

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Abstract

This protocol can be used on flash-frozen or RNAlater-preserved brain cortex. It is based on a previously published method (1) with a few modifications including, 1) Adding Recombinant RNase Inhibitor into the EZ prep lysis buffer in the incubation steps. 2) Passing the lysate through a 40 µm cell strainer after the tissue grinding. 3) Doing an additional wash. 4) Passing the nuclei suspension through a 20 µm filter before the final counting.

Materials

Reagents and devices

	A	B	C
	Item	Vendor	Part Number
	Nuclei Isolation Kit: Nuclei EZ Prep	Sigma	NUC101-1KT
	Recombinant RNase Inhibitor, 5,000 U, 40 U/µl	Takara	2313A
	10X Phosphate-Buffered Saline (PBS), molecular biology grade, 1L	Invitrogen	AM9625
	Bovine Serum Albumin (BSA), suitable for molecular biology, 100 mg	Sigma	B6917-100MG
	Cellometer ViaStain AO Staining Solution	Nexcelom Bioscience	CS1-0108-5ML
	Counting Chamber SD100, 2 counts/slide (75/BX)	Nexcelom Bioscience	CHT4-SD100-002
	KIMBLE Dounce tissue grinder set, 2 mL complete	Sigma	D8938
	40 µm cell strainer	VWR	21008-949
	20 µm sterile single-pack CellTrics filters	Sysmex	04-004-2325

Safety warnings

For hazard information and safety warnings, please refer to the MSDSs (Material Safety Data Sheets).

1 Prepare the following lysis buffers, wash buffer and resuspension buffer, and keep on ice. 15m

Lysis buffer1

	A	B
		1x
	Nuclei EZ lysis buffer	4 ml
	RNase Inhibitor	20 µl

Lysis buffer2

	A	B
		1x
	Nuclei EZ lysis buffer	4 ml
	RNase Inhibitor	4 µl

Wash buffer (PBS with 0.01% BSA and 0.04 U/µl RNase Inhibitor)

	A	B
		1x (ml)
	10x PBS	1
	2% BSA	0.05
	RNase Inhibitor	0.01
	H2O	8.94
	Total	10

Nuclei resuspension buffer (PBS with 1% BSA and 0.2 U/µl RNase Inhibitor)

	A	B
		1x (ml)
	10x PBS	0.1
	2% BSA	0.5
	RNase Inhibitor	0.005
	H2O	0.395
	Total	1

2 Add 2 mL of cold Lysis buffer1 into an empty douncing tube and keep on ice.

3 Drop the frozen tissue into the douncing tube and submerge it in the lysis buffer. Start immediately grinding the tissue with pestle A 25 times or until resistance disappears. Continue 15m

grinding with pestle B 25 times. Pass the lysate through a 40 µm filter into a new 50 mL tube on ice.

4 Rinse the douncing tube with the remaining 2 mL Lysis buffer1, pass through the same filter and into the same 50 mL tube.

5 Mix the lysate well by inverting the tube 5 times and incubate on ice for 00:05:00 . 5m

6 Pellet the nuclei by 500 x g, 4°C, 00:05:00 . Carefully aspirate the supernatant and set the nuclei pellet on ice. 5m

7 Resuspend the nuclei by adding 1 mL Lysis buffer2 and mix well with a P1000 pipette. Add the remaining 3 mL Lysis buffer2, mix well by inverting, and incubate on ice for 00:05:00 5m

8 Pellet the nuclei by 500 x g, 4°C, 00:05:00 as in step 6. Carefully aspirate the supernatant and set the nuclei pellet on ice. 5m

9 Resuspend the pellet with 750 µL Nuclei resuspension buffer, filter through a 20 µm filter, and collect into a 1.5 ml Eppendorf tube. Count the nuclei using Cellometer K2 (Nexcelom Bioscience) with the AO stain. Keep on ice until downstream processing.

Protocol references

1. Habib N, Li Y, Heidenreich M, Swiech L, Avraham-Davidi I, Trombetta JJ, et al. Div-Seq: Single-nucleus RNA-Seq reveals dynamics of rare adult newborn neurons. Science. 2016;353(6302):925-8.