

# Single nuclei isolation from OCT-embedded adult rat auditory cortex



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## About the CLEF Lab

The [CLEF \(Cortex, Learning, Epigenetics, & Function\) Laboratory](#) is a part of the Department of Psychology at Rutgers University. Their goal is to understand how the adult brain learns and remembers information from our daily and significant experiences. A core feature of their investigation is understanding the mechanisms of neuroplasticity, the brain's ability to rewire and reorganize, especially in the cortex, at the highest neural levels of representation.

### Notice

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## This protocol was used in

Gungor Aydin, A., Lemenze, A. & Bieszczad, K.M. Functional diversities within neurons and astrocytes in the adult rat auditory cortex revealed by single-nucleus RNA sequencing. *Sci Rep* 14, 25314 (2024). <https://doi.org/10.1038/s41598-024-74732-7>

## Materials

Material	Supplier	Part Number	Notes
2-methyl-butane (Isopentane)	Sigma-Aldrich®	277258	or equivalent
Optimal cutting temperature medium (OCT)	Sakura Finetek or equivalent	4583	or equivalent
Tris-HCl	Millipore Sigma®	T2194	or equivalent
NaCl	Millipore Sigma	59222C	or equivalent
MgCl <sub>2</sub>	Millipore Sigma	M1028	or equivalent
Nonidet® P40 (NP-40)	Millipore Sigma	74385	or equivalent
Nuclease-free Water	Thermo Fisher Scientific®	AM9922	or equivalent
PBS, pH 7.4	Gibco®	10-010-031	or equivalent
BSA	Thermo Fisher Scientific	AM2616	or equivalent

Centrifuge with swinging bucket rotor	Varies	Varies	Compatible with 15 mL or 5 mL centrifuge tubes and capable of reaching 4°C.
1.5 ml microcentrifuge tubes	Fisherbrand®	05-408-130	or equivalent
1.5 ml Protein LoBind®	Eppendorf®	022431081	or equivalent
Plastic pestle homogenizer	Fisherbrand	12-141-364	or equivalent
30 µm strainer	pluriSelect®	435003003	or equivalent
RNaseZAP® RNase Decontamination Solution	Thermo Fisher Scientific	AM9780	
Nuclei Isolation Column			

## Lysis Buffer

Reagents	Stock Concentration	Final Concentration	Volumes for 10 mL
Tris-HCl	10 mM	10 mM	100 $\mu$ l
NaCl	5 M	10 mM	20 $\mu$ l
MgCl <sub>2</sub>	1 M	3 mM	30 $\mu$ l
Nonidet P40 (NP-40)	10%	0.1%	100 $\mu$ l
Nuclease-free Water	-	-	9.75 mL

## Nuclei Wash and Resuspension Buffer

Reagents	Stock Concentration	Final Concentration	Volumes for 5 mL
PBS	1X	-	3.975 mL
BSA	5%	1%	1 mL
0.2 U/ $\mu$ l RNase inhibitor	40X	0.2X	25 $\mu$ l

## Preparation

1. Clean the bench top and dissection area with 70% ethanol, followed by RNaseZAP to remove RNases.
2. Tissue Collection
  - a. Rapidly remove rat brains and flash-freeze in pre-chilled 2-methylbutane (dry ice or liquid nitrogen) for 60 seconds, then store at -80°C until sectioning.

- b. Embed brains in optimal cutting temperature medium (OCT) and section with a cryostat (250  $\mu\text{m}$  sections).
- c. Micropunch sections containing the auditory cortex (AC) with a 1 mm diameter micropuncher.
- d. Transfer to ice-cold centrifuge tubes and store at  $-80^{\circ}\text{C}$  until nuclei isolation.

## Procedure



**Note:** Keep all tools, materials, and reagents on ice. Pre-cool the swinging bucket centrifuge to  $4^{\circ}\text{C}$

1. Combine frozen micropunches and briefly thaw on wet ice.
2. Transfer to a 1.5 mL tube with 400  $\mu\text{l}$  chilled lysis buffer.
3. Dissociate the tissue on ice using a plastic pestle homogenizer to grind the tissue manually until homogeneous.
4. Incubate on ice for 10 minutes.
5. Pass micropunches through pre-chilled Nuclei Isolation Column\* (spin column) by centrifuging at 500 x g for 3 minutes at  $4^{\circ}\text{C}$  in a swinging bucket centrifuge to pellet nuclei.
6. Remove the supernatant without disrupting the pellet.
7. Wash nuclei-pellets with 1 mL chilled Nuclei Wash and Resuspension Buffer by gently pipette mixing 10 times.
8. Filter nuclei through a 30  $\mu\text{m}$  strainer into a 1.5 mL centrifuge tube (Protein LoBind tube highly recommended)
9. Centrifuge at 500 x g for 3 minutes at  $4^{\circ}\text{C}$  in a swinging bucket centrifuge to pellet nuclei.
10. Remove the supernatant without disrupting the pellet.

11. Resuspend nuclei in 500  $\mu$ L Nuclei Wash and Resuspension Buffer by gently pipetting 10 times.
12. Filter twice through a 30  $\mu$ m strainer, collecting the filtrate in a new tube each time.
13. Count nuclei using a preferred accurate method.



**Note:** In our experience, nuclei are much stickier than whole cells.

For researchers making the single-nuclei suspension for the first time, we suggest confirming that the sample contains mostly single nuclei. To do this, take 10  $\mu$ L of the sample and stain it with Hoechst, DAPI, or AOPI for more than 5 min, place it on a hemocytometer, and visualize it under a 20X and 40X objective on an epifluorescence microscope. A best practices resource for hemocytometer use, see: <https://www.hemocytometer.org/>

14. Aliquot nuclei and centrifuge at 200 x g for 10 min at 4  $^{\circ}$ C.
15. Proceed to the appropriate Parse Biosciences Nuclei Fixation Kit [User Guide](#) (either standard Nuclei Fixation, Low-Input Nuclei Fixation).

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