May 21, 2025 Version 2

Isolation of nuclei from fresh frozen brain sections V.2

DOI
https://dx.doi.org/10.17504/protocols.io.dm6gp9qjdvzp/v2

Simona Sarafinovska[1], Allen Yen[1], Sneha Chaturvedi[1], Emma Jones[1], Kelli McFarland White[1]

[1]Washington University School of Medicine

Rebecca Chase
Washington University Medical School in St. Louis

DOI: https://dx.doi.org/10.17504/protocols.io.dm6gp9qjdvzp/v2

Protocol Citation: Simona Sarafinovska, Allen Yen, Sneha Chaturvedi, Emma Jones, Kelli McFarland White 2025. Isolation of nuclei from fresh frozen brain sections. **protocols.io** https://dx.doi.org/10.17504/protocols.io.dm6gp9qjdvzp/v2Version created by **Rebecca Chase**

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

Created: May 21, 2025

Last Modified: May 21, 2025

Protocol Integer ID: 218705

Keywords: nuclei isolation, iodixanol, fresh frozen brain sections, homogenization, snRNAseq, fresh frozen brain sections this protocol, isolation of nuclei, fresh frozen brain section, isolation with an iodixanol, isolation, nuclei, brain section, iodixanol

Abstract

This protocol is for the isolation of nuclei from fresh frozen brain sections that have been placed on slides. It's main steps consist of homogenization, washing, and isolation with an iodixanol. Once nuclei have been isolated, they should be processed according to the manufacturer's protocol for sequencing.

Materials

Bleach
RNaseZap
Wet Ice
Dry Ice
Low-retention tips
Low-retention tubes
Ultracentrifuge
Swinging-bucket tabletop centrifuge
Dounce homogenizer
Millipore water
Aluminum foil
2mL lo-bind Eppendorf tube
4mL centrifuge tube
15mL low-retention centrifuge tube
1x DPBS
40um filter
Trypan or AO/PI

Protocol materials

RNase Inhibitor **Promega Catalog #**N2515

Before start

IMPORTANT: primate and human brain tissue are potentially infectious. Collect all contaminated instruments, discarded tissue, and nuclei in a beaker to be bleached at the end of the experiment. Decontaminated tubes and tips go into a biohazard waste box.

Clean all working surfaces and pipettes with bleach and RNaseZap. Regularly clean gloves with RNaseZap.

Keep all the solutions and materials on wet ice. Keep the brain samples on dry ice.

All samples should be handled equally at all steps, as different cell types might have nuclei more or less vulnerable to physical dissociation. All pipetting and resuspension steps should be gentle and consistent to avoid nuclei shearing and clumping.

Use **low-retention** tips and tubes for every step where nuclei are involved.

Solutions

1 Prepare solutions on the day of experiment.

1.1 **Homogenization Buffer**

maintain at 4 °C

	Reagent	Stock	Final	Amount-4 Samples (uL)	Stock Temp
	Tris-HCl (pH 7.4)	1M	10mM	120	RT
	KCl	2M	25mM	150	RT
	MgCl2	1M	5mM	60	RT
	DTT	1M	1mM	12	-20ºC
	RNAse Inhibitor	40U/mL	0.2U/uL	60	-20ºC
	Protease Inhibitor	Dissolve 1 tablet in 600uL nuclease-free water	-	2 tablets	-20ºC
	Nuclease-free water	-	-	Fill to 12mL	RT

RNase Inhibitor **Promega Catalog #**N2515

Protease Inhibitor **Roche Catalog #**4693159001

1.2 **Nuclei Wash Buffer**

maintain at 4 °C

	Reagent	Stock	Final	Amount-4 Samples	Stock Temp
	DPBS (Ca2+/Mg2+ free)	1x	1x	28.8mL	RT
	BSA	10%	1%	3.2mL	-4ºC
	RNase Inhibitor	40U/mL	0.2U/uL	160uL	-20ºC

RNase Inhibitor **Promega Catalog #**N2515

1.3 **50% Iodixanol - can be prepped during first spin**

maintain at 4 °C

	Reagent	Amount-4 Samples (mL)	Stock Temp
	OptiPrep (60% Iodixanol)	6	-4ºC
	DPBS (Ca2+/Mg2+ Free)	1	RT

1.4 **25% Iodixanol**

maintain at 4 °C

	Reagent	Amount-4 Samples (mL)	Stock Temp
	50% Iodixanol	2	-4ºC
	DPBS (Ca2+/Mg2+ free)	2	RT

Preparation

2 Before starting - do the following

2.1 Turn on benchtop centrifuge with 2 mL swinging-bucket inserts for spins to 4 °C

2.2 Clean 2 mL dounce homogenizer and pestles with bleach, rinse with reagent grade water, wash with RNAseZap, and rinse with Millipore water. Wrap top of dounce homogenizer and bottom of pestles with aluminum foil.

2.3 Prepare working solutions go to step #1

Add pre-chilled 500 µL homogenization buffer to the dounce homogenizer and keep on wet ice.

Slide Scraping

3 Use cell scraper to remove tissue from the 4 100 µm slides. Minimize OCT contamination.

> Note
>
> Place finger behind slide to warm up tissue and make it easier to scrape.

4 Use glass Pasteur pipette to remove tissue from cell scraper and place into dounce.

5 Spin to remove tissue and leave pipette in dounce to soak.

6 After scraping all 4 slides, rinse inside of glass pipettes with 500 µL homogenization buffer to remove any remaining nuclei.

Isolation

7 Homogenize brain tissue. Repeat for all tissue samples.

7.1 15 strokes On ice with pre-chilled 'A' pestle and 15 with 'B' grinder.

> Note
>
> Minimize bubbles by not removing the pestle from the liquid while homogenizing.

7.2 Transfer brain lysate into 2 mL lo-bind Eppendorf tube.

7.3 Add 1 mL homogenization buffer to glass homogenizer and transfer remaining lysate to same Eppendorf tube. Keep tube On ice

8 Swing bucket centrifuge 500 x g, 4°C , 5 minutes

9 Carefully aspirate the supernatant. Resuspend each pellet to a final volume of 1 mL in homogenization buffer.

10 Add 1 mL of 50% Iodixanol to samples.

Expected result

2 mL 25% Sample/Iodixanol layer.

Note

Vortex the iodixanol before pipetting.
Weigh tubes to ensure level.

11 Centrifuge 10000 x g, 4°C , 30 minutes with minimal acceleration and deceleration ramp rate.

12 With a wide-bore P1000 tip, remove myelin debris from top layer of tube.
Aspirate supernatant leaving behind nuclei pellet and approximately 500 µL of buffer.

13 Resuspend with 1.5 mL of Nuclei Wash Buffer. Gently mix by inverting or pipetting

14 Centrifuge 500 x g, 4°C , 5 minutes

15 Carefully remove and discard the supernatant.

16 Resuspend nuclei in 1 mL of Nuclei Wash Buffer.

17 Centrifuge 500 x g, 4°C , 5 minutes

18 Carefully remove and discard the supernatant.

19 Resuspend nuclei in 1 mL 1x dPBS.

20 Pass through 100 µm pluristrainers into new 2 mL lo-bind tube.
Wash filter with 200 µL dPBS to remove any additional stuck nuclei.
Keep on ice.

21 Remove 10 µL into PCR strip, stain with 10 µL AO/PI (1:1) and pipette mix. Load 20 µL on K2 slide and count with MGI Nuclei with Debris settings.

22 Proceed directly to downstream protocol (i.e. nuclei fixation of molecular technology).

Protocol references

Del-Aguila JL, Li Z, Dube U, Mihindukulasuriya KA, Budde JP, Fernandez MV, Ibanez L, Bradley J, Wang F, Bergmann K, Davenport R, Morris JC, Holtzman DM, Perrin RJ, Benitez BA, Dougherty J, Cruchaga C, Harari O. A single-nuclei RNA sequencing study of Mendelian and sporadic AD in the human brain. Alzheimers Res Ther. 2019 Aug 9;11(1):71. doi: 10.1186/s13195-019-0524-x. PMID: 31399126; PMCID: PMC6689177.