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Isolation of nuclei from fresh frozen brain sections V.1

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

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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

Protease Inhibitor **Roche Catalog #**4693159001

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	38.8mL	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**

maintain at 4 °C

	Reagent	Amount-4 Samples (mL)	Stock Temp
	OptiPrep (60% Iodixanol)	17.5	-4ºC
	DPBS (Ca2+/Mg2+ Free)	3	RT

1.4 **35% Iodixanol**

maintain at 4 °C

	Reagent	Amount-4 Samples (mL)	Stock Temp
	50% Iodixanol	7	On ice
	DPBS (Ca2+/Mg2+ free)	3	RT

Preparation

2 Before starting - do the following

2.1 Turn on ultracentrifuge for gradient
Set to 4 °C and ensure that decelerations are set to no brake (RCF: 9999, 30min)

2.2 Get Beckman S0410 Rotor (Swinging Bucket) from cold room
Turn on swinging-bucket tabletop centrifuge for spins to 4 °C and ensure that decelerations are set to no brake

Note

Leave note when in use.

2.3 Clean dounce homogenizer and centrifuge tubes with bleach, rinse with reagent grade water, wash with RNAseZap, and rinse with Millipore water. Finally, wrap top of tube and

bottom of homogenizers with aluminum foil.

2.4 Prepare working solutions go to step #1

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

Isolation

3 Transfer brain tissue to homogenizer.

4 Homogenize brain tissue. Repeat for all tissue samples.

4.1 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.

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

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

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

6 While centrifuging, add 2 mL of 35% Iodixanol to a centrifuge tube (4 ml, thin-wall polystyrene tube) for each sample.

> Note
>
> Vortex the iodixanol before pipetting.

7 Carefully aspirate the supernatant and resuspend each pellet to a final volume of 1 mL in homogenization buffer.

8 Add 1 mL of 50% iodixanol to each sample.

Note

Vortex the iodixanol before pipetting.

This will create 25% iodixanol with the nuclei samples.

9 Pipette the samples up and down with a low retention pipette tip and carefully layer the sample on top of the 35% iodixanol solution.

Note

It is important that the layers do not mix.

10 Weigh the tubes and adjust the volumes to match the heaviest within 0.1 mg

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

12 Carefully remove the centrifuge tubes.
The nuclei should be visible as a cloudy layer in the interface of the 25:35% iodixanol.

13 Remove the nuclei from the interface with a low-retention P1000 tip.
Transfer up to 2 mL to a 15mL low-retention centrifuge tube.

14 Fill up to 6 mL with Nuclei Wash Buffer and gently mix by inverting or pipetting.

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

16 Carefully remove and discard the supernatant.

17 Resuspend nuclei in 1 mL of Nuclei Wash Buffer.

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

19 Resuspend nuclei in 1 mL 1x dPBS and pass them through a 40 µm filter.

20 Stain with Trypan or AO/PI (1:1) and count on hemacytometer or using a fluorescent microscope or Countess FL Automated Cell Counter.
Check the morphology, size, and absence of nuclei clumps or tissue debris.

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

Protocol references

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