JIN LAB Nuclei extraction protocol (from perinatal mouse brain)

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NOTES

Optimal douncing and lysis conditions depend on the specific tissue used and on intended downstream processes. Amount of strokes using dounce pestles A or B, post-douncing lysis rest, and formulation of NIB-NP and NIB-BSA may all be optimized for different tissues. Those conditions here are intended for fresh P3-P4 mouse cortex, a fragile specimen.

MATERIALS

Dounce Homogenizer (Sigma-Aldrich D8938)
70um Cell Strainers (Corning 352350)

Ultra Pure Water (Fisher Scientific RGF-3410)
10X PBS (Life Technologies AM9625)
NIM (Nuclei Isolation Medium) (fresh or can be stored overnight at 4C)

- ➔ Sucrose solution (1.5M, or make fresh from solid) (Sigma-Aldrich 84097)
- ➔ KCl (2M) (Life Technologies AM9640G)
- ➔ HEPES (1M, pH 7.5) (ThermoFisher 15630080)
- ➔ $MgCl_2$ (1M) (Life Technologies AM9530G)

NIB-NP (Nuclei Isolation Buffer with NP40) (fresh)

- ➔ NIM
- ➔ Dithiothreitol (DTT) (1M) (Sigma-Aldrich 646563)
- ➔ Kollidon, or Poly(1-vinylpyrrolidone-co-vinyl acetate) (Sigma-Aldrich 190845)
- ➔ Nonidet P40 (NP-40), or substitute/alternative (10% v/v) (MilliporeSigma 492016)

NIB-BSA (Nuclei Isolation Buffer with BSA) (fresh)

- ➔ NIM
- ➔ DTT
- ➔ Bovine Serum Albumin (BSA) (solid or 10% w/v) (Sigma-Aldrich B6917)

METHODS

1. If need to prepare NIM, thaw or prepare **1.5M Sucrose** according to **Table 1**.
    a. Once dissolved, filter with sterile 0.22um filter. Excess can be aliquoted and stored at -20C. Check for white bacterial growth before using thawed sucrose.

Table 1: Sucrose Prep (more than ⅙ as much as NIM, filtration will cause loss)

Final [Sucrose] (M)	Mass Sucrose (g)	Total Volume (mL)
1.5	20.538	40

2. Prepare **NIM** according to **Table 2**. Vortex to mix and then filter with 0.22um filter.

Table 2: NIM Prep (6mL per sample preparation; more for 1 sample - filtration will cause loss)

Reagent		Final Concentration (mM)	Amount to Add	
Name	Initial Concentration (M)		#	Units
Sucrose	1.5	250	1	mL
KCl	2	25	75	**uL**
HEPES	1	10	60	**uL**
MgCl2	1	5	30	**uL**
Water to fill			4.835	mL
Total			6	mL

3. Thaw or dissolve DTT and BSA.
4. Prepare **NIB-NP** and **NIB-BSA** on ice according to **Tables 3** and **4**, keep chilled. Dissolving Kollidon may take some time.

Table 3: NIB-NP Prep (4mL per sample preparation)

Reagent	Initial Concentration		Final Concentration		Amount to Add	
	Amount	Units	Amount	Units	#	Units
NIM					3.956	mL
DTT	1	M	1	mM	4	**uL**
Kollidon	100	% (solid)	10	mg/mL	40	**mg**
NP-40	10	% (liquid)	0.1	%	40	**uL**
Total					4	mL

Table 4: NIB-BSA Prep (at least 2mL per sample preparation)

Reagent	Initial Concentration		Final Concentration		Amount to Add	
	Amount	Units	Amount	Units	#	Units
NIM					1798	uL
DTT	1	M	1	mM	2	uL
BSA*	10	% (liquid)	1	%	200	uL
Total					2	mL

*Prepare 10% by dissolving 0.1g solid filled up to 1mL water

5. Fill dounce vials with NIB-NP to the line (~1.6mL) and embed in ice (past the fill level). Embed centrifuge tubes in ice. Ensure the centrifuge is cooling to 4C. Nuclei should always be kept at 4C/on ice.
6. Transfer tissue to dounce vial and dounce 3x with pestle A, then 3x with pestle B.
    a. Do not remove vials from ice to dounce. Bring rods only close to bottom of vials or gently touch it, do not add pressure or grind.
    b. The number of douncing and post-dounce lysis rest time will need to be adjusted for different specimen types (age, tissue types, fresh versus frozen samples, etc).
7. Transfer the lysis to a fresh tube, add 2 mL fresh NIB-NP, and continue lysing for 5 minutes resting embedded in ice.
8. Transfer the sample through a pre-wetted, 70 um strainer to a centrifuge tube.
    Note: pre-wet the strainer with PBS to prevent sample loss.
9. Centrifuge at 200g at 4C for 8 minutes.
    Note: shorter centrifugation time may lead to loss of nuclei.
10. Carefully remove supernatant without disrupting pellet, then resuspend by thoroughly but gently pipetting in 0.5mL **NIB-BSA**.
11. Repeat centrifugation and resuspension in step 10. Sample is now ready for FACS or other analysis. Check the nuclei morphology under the scope to ensure the integrity of the sample.