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Human Spinal Cord Nuclei Isolation

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Abbas Rizvi[1], Elena Kandror[1], Tom Maniatis[1]

[1][Zuckerman Mind Brain Behavior Insitute, Columbia University]

Human Cell Atlas Metho...

Abbas Rizvi
Columbia University

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Protocol status: Working
This simple protocol enables rapid isolation of mouse and human spinal nuclei.

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Materials

MATERIALS

OptiPrep™ Density Gradient Medium **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D1556)

Troubleshooting

Buffer Prep

1 **10x Stock Salt Solution:** (raise to 50mL in water)
note: can be stored at 4 °C *for up to 2 weeks*

		Final Concentration
2.5 mL	CaCl2 (1M)	50mM
1.5 mL	Magnesium Acetate (1M)	30mM
5 mL	Tris pH7.8 (1M)	100mM
35 µL	2-Mercaptoethanol (14.3M)	10mM

2 **50% Optiprep:** (raise to 50mL in water)
note: can be stored at 4 °C *for up to 2 weeks*

25 mL	Optiprep
5 mL	10x Stock Salt Solution
8 mL	Sucrose (2M)

3 **29% Optiprep:** (raise to 50mL in water)
note: can be stored at 4 °C *for up to 2 weeks*

14.5 mL	Optiprep
5 mL	10x Stock Salt Solution
8 mL	Sucrose (2M)

4 **1x Homogenization Buffer:** (raise to 10mL in water)
note: prepare fresh each time, keep on ice

		Final Concentration
1 mL	10x Stock Salt Solution	1X
1.6 mL	Sucrose (2M)	320mM
200 µL	EDTA (5mM)	0.1mM
100 µL	NP-40 (10%)	0.1%
10 µL	PMSF (100mM)	100uM
5 µL	DAPI (5mg/ml) -- optional	

Homogenization

5 ***Critical Note: Perform all steps on ice!*** 0 °C

Add 4 mL **1x Homogenization Buffer** into a clean 7ml capacity dounce homogenizer
note: if using NeuN antibody, add it here (1:500 final dilution = 20ul primary labeled NeuN antibody)

6 Cut 4 frozen tissue slices ~0.2mm thick and transfer into dounce homogenizer.

7 Homogenize tissue with:
15 strokes loose pestle
20 strokes tight pestle
(avoid bubbles)

8 Filter the sample through a 100um mesh into a fresh 15ml tube; rinse the filter with 1 mL **1x Homogenization Buffer** and collect in the same tube.

9 Incubate 00:10:00

10 Add equal volume **50% Optiprep** (~ 4.5 mL)

11 Invert thoroughly but gently to mix (avoid bubbles). This becomes a 25% optiprep solution.

12 Gently layer over 10 mL **29% Optiprep** in swinging bucket ultracentrifuge compatible tube (we use Beckman #344058)

13 Spin in a swinging bucket centrifuge at 10,100g (=7400rpm in SW-28 Beckman rotor) for 00:30:00 at 4 °C
note: acceleration and break both on at max (setting 9)

Once the spin is done, the nuclei will be pelleted at the bottom of the ultracentrifuge tube. However, don't disrupt the gradient as doing so can introduce myelin contaminant into the sample!

Nuclei Resuspension

14 Going from the top of the solution, remove the 25% optiprep layer using a 1ml pipet.
note: if there is myelin coating over the top of the solution, remove it first.

15 Once the 25% optiprep layer is mostly removed, continue to pipet out solution from the top of the tube. The most effective way to do this while preventing myelin carry-over is to "slurp" the solution with a 1ml pipet such that you are effectively aspirating bubbles (50% solution and 50% air).

16 Once there is ~5ml solution left, pipet the solution out normally, 1ml at a time. Do not reuse the pipet tip. Remove the entire volume, being very careful with the last aspiration so as not to disturb the nuclei.

17 Resuspend the nuclei in your buffer of choice (eg, 1mL of PBS + 0.1% BSA) by forcefully expelling the buffer directly against the bottom of the ultracentrifuge tube. Pipet up and down to fully resuspend the nuclei, and transfer to an ice-cold 1.5mL tube.