Isolation and Fixation of Nuclei from the Mouse Brain for Dip-C

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Reagents

1. Prepare 1.5 M sucrose (40 mL):
   - 20.538 g Aldrich Catalog #84097 (final: 1.5 Molarity (M), 51.3 Mass / % volume)
   - 40 mL water
   - Heat and vortex to mix.
   - Filter to sterilize.
   - Store at 4 °C.

2. Prepare Nuclei Isolation Medium 1 (45 mL; note that Tris is replaced with HEPES):
   - 7.5 mL 1.5 M sucrose (final: 250 Milimolar (mM), 8.56 Mass / % volume)
   - 562.5 µl KCl (2 M), RNase-free Thermo (final: 25 Milimolar (mM))
   - 450 µl HEPES (1 M) Thermo (final: 10 Milimolar (mM))

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225 µl 1 M MgCl₂ Invitrogen - Thermo
- 36.2625 mL water
- Vortex to mix.
- Store at 4 °C.

3 Prepare 1% BSA in PBS (1.2 mL per sample; recipe below for 10 mL):
- 0.1 g Bovine Serum Albumin (BSA) Fraction V—Molecular Biology Grade Gemini Bio-Products Catalog #700-106P
- 10 mL PBS, pH 7.4 Thermo
- Vortex to mix.
- Rotate until fully dissolved.
- Chill on ice.
- Store at -20 °C if needed.

Nuclei Isolation

4 Chill a KIMBLE 2mL Glass Dounce Tissue Grinder
Set Sigma Catalog #D8938 (or larger sizes: KIMBLE Dounce tissue grinder set 7 mL complete Sigma Aldrich Catalog #D9063, KIMBLE Dounce tissue grinder set 15 mL complete Sigma Aldrich Catalog #D9938, KIMBLE Dounce tissue grinder set 40 mL complete Sigma Aldrich Catalog #D9188, or KIMBLE Dounce tissue grinder set 100 mL complete Sigma Aldrich Catalog #D0189)

5 Prepare 1 mM DTT:
- 1 mL water
- 1 µl 1M DL-Dithiothreitol solution (DTT) Sigma
- Vortex to mix.

6 Prepare Nuclei Isolation Buffer without Triton (6 mL per sample):
- 6 mL Nuclei Isolation Medium 1
- 6 µl 1 mM DTT (final: 5 Micromolar (µM))
- Vortex to mix.
- Chill on ice.

7 Prepare Nuclei Isolation Buffer with Triton (2 mL per sample):

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8 Add 2 mL ice-cold Nuclei Isolation Buffer with Triton to the homogenizer.

9 Dounce the tissue with 5 strokes of the loose pestle (A), and 15 strokes of the tight pestle (B).

10 Transfer the homogenate to a tube.

11 Centrifuge at 100 x g, 4°C, 00:08:00.

12 Carefully remove supernatant without disrupting the soft pellet.

13 Resuspend in 2 mL Nuclei Isolation Buffer without Triton.

14 Centrifuge at 100 x g, 4°C, 00:08:00.

15 Carefully remove supernatant without disrupting the soft pellet.

16 Resuspend in 2 mL Nuclei Isolation Buffer without Triton.

17 Filter with Falcon 40 µm Cell Strainer Corning Catalog #352340 or Corning™ Falcon™ Test Tube with 35µm Cell Strainer Snap Cap Corning Catalog #352235.

18 Fixation

To every 1 mL of cells, add 66.7 µl.
19. Rotate at room temperature for 00:10:00.

20. Add 200 µl 1% BSA in PBS.

21. Invert to mix.

22. Centrifuge at 1000 x g, 4°C, 00:05:00.

23. Remove supernatant.

24. Resuspend in 1 mL ice-cold 1% BSA in PBS.

25. Count with C-Chip disposable hemacytometer INCYTO Catalog #DHC-N01, and aliquot to up to 0.5 million cells per tube.

26. Centrifuge at 1000 x g, 4°C, 00:05:00.

27. Remove supernatant.

28. Store at ~80 °C.