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

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1 Works for me dx.doi.org/10.17504/protocols.io.bpsxmnfn


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Reagents

1 Prepare 1.5 M sucrose (**40 mL**):

sucrose **Sigma**

- **20.538 g** **Aldrich Catalog #84097** (final: [M] **1.5 Molarity (M)** , [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: [M] **250 Milimolar (mM)** , [M] **8.56 Mass / % volume**)

KCl (2 M), RNase-free **Thermo**

- **562.5 µl** **Fisher Catalog #AM9640G** (final: [M] **25 Milimolar (mM)**)

HEPES (1 M) **Thermo**

- **450 µl** **Fisher Catalog #15630080** (final: [M] **10 Milimolar (mM)**)

1M MgCl2 **Invitrogen - Thermo**

- **225 µl** **Fisher Catalog #AM9530G** (final: **5 Milimolar (mM)**)
- **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**

  PBS, pH 7.4 **Thermo**
- **10 mL** **Fisher Catalog #10010023**
- Vortex to mix.
- Rotate until fully dissolved.
- Chill **On ice** .
- Store at **-20 °C** if needed.

Nuclei Isolation

4 KIMBLE 2mL Glass Dounce Tissue Grinder

Chill a 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**) **On ice** .

5 Prepare 1 mM DTT:

- **1 mL** water

  1M DL-Dithiothreitol solution (DTT) **Sigma**
- **1 µl** **Aldrich Catalog #646563** (aliquoted and stored at **-20 °C**)
- 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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- **2 mL** Nuclei Isolation Buffer without Triton
  Triton X-100, 10% solution
- **20 µl** **Sigma Catalog #93443** (final: **0.1 % (v/v)**)
- Vortex to mix.
- Chill **On ice** .

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

Fixation 10m

18 To every **1 mL** of cells, add **66.7 µl**

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Paraformaldehyde 32% (methanol free) **Electron Microscopy Sciences Catalog #15714** (final: **2 Mass Percent**).

19 Rotate at **Room temperature** for **00:10:00** . 10m

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 C-Chip disposable
Count with 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** .

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