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Village Nuclei Isolation With Myelin Removal

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ABSTRACT

Isolation of nuclei from fresh-frozen brain tissue from sets of multiple (typically 2-20) human donors for analysis as a “cell village” (Wells et al., PMID 36796362) in which nuclei from all donors are analyzed together. Adapted from dx.doi.org/10.17504/protocols.io.2srged6 and dx.doi.org/10.17504/protocols.io.bq64mzgw

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

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Before Starting

1 Gather Supplies

- Razor Blades
- Glass slides
- Syringes with needles (3 mL syringe with 26 1⁄2 gauge needle)
- Myelin removal beads (cat # 130-096-731) https://www.miltenyibiotec.com/US-en/products/myelin-removal-beads-ii-human-mouse-rat.html#gref
- Eppendorf tubes (1.5 mL and 5 mL)
- Eppendorf or Rainin pipette tips
- Dry ice
- 20 µM filter
- 6 well tissue culture plate
- Magnetic Bead Separation Rack for 1.5 mL tubes
- RNAse free water
- PBS
- BSA
- Lucigen RNAse inhibitor (i)

1.1 Prep

- Turn on and chill 1.5 mL and 50 mL centrifuges with swinging buckets to 4°C
- Prepare all the reagents needed on ice
- Clean glass slides with ethanol
- Pre-chill glass slides, razors, and syringes with needles

1.2 Solutions to make fresh before starting experiment

- DB + 5% Kollidon 64 + 0.2 U/uL Lucigen RNAse Inhibitor (5 uL/mL)
- Filtering and spinning down buffer: DB + 0.016 U/uL Lucigen RNAse inhibitor (5-12.5 uL/12.5 mLs)
  (In earlier experiments 12.5 uL of inhibitor were used, but this was later scaled down to 5

uL)

- 10X cell loading buffer: 1% BSA + 0.2 U/uL of i

Tissue Homogenization

2 Section and mince tissue using a pre-chilled razor and glass slide in cryostat or on ice. Avoid including white matter as much as possible.

3 Transfer minced tissue into a well of the 6-well plate containing either 1-2 mLs of extraction buffer if doing a small extraction (~600 total mg of minced tissue) or 3+ mLs of extraction buffer if doing a larger extraction (~1000 total mg of minced tissue). Mix ~20 times with 1000µL pipette.

4 Incubate sample on ice for 10 minutes, mixing ~20 times with the pipette about every 2 minutes.

5 Syringe the sample twice.

5.1 If doing a small extraction (1-2 mLs), syringe into the same well of a 6-well tissue culture plate.

5.2 If doing a larger extraction (3+ mLs), transfer the syringed tissue+buffer into a new well of a 6-well tissue culture plate each time you pass the sample through a syringe.

6 Filter through a 20 uM filter, flushing with DB + i (flush the filter with 12.5 mLs DB + i for every mL of extraction buffer used).

6.1 Split the volume into multiple 50 mL tubes as needed so that each tube has 12.5 mL of sample. (For a 1 mL extraction, 12.5 mLs will go into 1 tube; for a 4 mLs extraction, a total volume of 50 mLs will go into 4 tubes with 12.5 mLs per tube.)

7 Spin down for 10 minutes at 4ºC - 500 x g for human/primate tissue or 600 x g for mouse.

8 Remove and discard supernatants.

9 Combine and resuspend the pellets in DB + i. (Use 10 mLs DB + i for a large ~1000 mg extraction.)

10 Count a dilution of the nuclei.

Myelin Removal

11 For myelin removal, dilute nuclei to 1000 n/µL with 10X cell loading buffer (1% BSA in PBS + 0.2U/uL i).

12 Add myelin removal beads (10 uL of beads per mL of nuclei at 1000 N/uL).

13 Mix and aliquot nuclei into 1.5 mL tubes.

14 Incubate nuclei with rotation at 4ºC for 15 minutes.

15 Briefly spin down the tubes to get liquid off the caps, then place on a magnet at 4ºC for 30 minutes.

16 Transfer nuclei to new tubes, pulling up from the bottom and avoiding the myelin pellet on the side of the tube.

17 Spin down the nuclei at 400 x g at 4ºC for 5 minutes.

18 Remove and discard the supernatant.

19 Resuspend the pellet in 10X cell loading buffer (1% BSA + 0.2 U/uL i).

20 Count a dilution of the nuclei.