



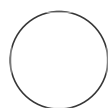
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Village Nuclei Isolation With Myelin Removal V.2

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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

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MANUSCRIPT CITATION:

Wells et al., PMID 36796362

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nuclei, nuclei extraction,
snRNAseq, RNAseq, human
tissue, brain tissue, multiple
donors

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 1000 μ L 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 μ M 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 4°C - 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/μL i).
- 12 Add myelin removal beads (10 μL of beads per mL of nuclei at 1000 N/μL).
- 13 Mix and aliquot nuclei into 1.5 mL tubes.
- 14 Incubate nuclei with rotation at 4°C for 15 minutes.

- 15** Briefly spin down the tubes to get liquid off the caps, then place on a magnet at 4°C 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 4°C 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.