



OCT 30, 2023

OPEN ACCESS



**DOI:**  
[dx.doi.org/10.17504/protocols.io.4r3l22e3xl1y/v1](https://dx.doi.org/10.17504/protocols.io.4r3l22e3xl1y/v1)

**Protocol Citation:** Steve McCarroll, Emi Ling, Melissa Goldman, Nora Reed 2023. Village Nuclei Isolation With Myelin Removal.  
**protocols.io**  
<https://dx.doi.org/10.17504/protocols.io.4r3l22e3xl1y/v1>

**MANUSCRIPT CITATION:**  
**Wells et al., PMID 36796362**

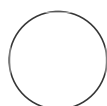
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## Village Nuclei Isolation With Myelin Removal

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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](https://dx.doi.org/10.17504/protocols.io.2srged6) and [dx.doi.org/10.17504/protocols.io.bq64mzgw](https://dx.doi.org/10.17504/protocols.io.bq64mzgw)

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

**Created:** Oct 30, 2023

**Last Modified:** Oct 30, 2023

**PROTOCOL integer ID:**  
90143

**Keywords:** fresh-frozen  
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 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 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/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 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.