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# Isolation of nuclei from frozen human skeletal and cardiac muscle for single nucleus RNA and chromatin assays V.2

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This protocol was adapted for the isolation of single nuclei from frozen skeletal and cardiac muscle tissues for molecular characterization with the SNARE-seq2, sci-ATAC-seq, and snRNA-seq assays.

References:
(1) Preissl et al (2015). Circulation Research. Doi: 10.1161/CIRCRESAHA.115.306337
(2) Hocker et al (2021). Science Advances. Doi: 10.1126/sciadv.abf1444

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protocol

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Required consumables per sample:

1. gentleMACS M Tube (Miltenyi Biotec, 130-096-335)
2. CellTrics filter, 30 uM (CellTrics, 04-0042-2316)
3. Eppendorf Tubes, 5 mL

Required instrument:

1. gentleMACS Tissue Dissociator (Miltenyi Biotec, 130-095-937)

Buffers:

| A | B | C | D | E | F |
|---|---|---|---|---|---|
| **Reagent** | **Stock conc.** | **Final conc.** | **1 mL** | **5 mL** | **10 mL** |
| PBS | 1X | 1X | 0.925 mL | 4.625 mL | 9.25 mL |
| BSA (Sigma Aldrich) | n/a | 5% | 50 mg | 250 mg | 500 mg |
| IGEPAL CA-630 / NP 40 | 10% | 0.20% | 20 uL | 100 uL | 200 uL |
| DTT | 100 mM | 1 mM | 10 uL | 50 uL | 100 uL |
| Protease Inhibitor | 25X | 1X | 40 uL | 200 uL | 400 uL |
| Enzymatics RNase In | 40 U/uL | 0.2 U/uL | 5 uL | 25 uL | 50 uL |

**Nuclear Permeabilization Buffer (NPB). Make fresh for each use.**



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| A | B | C | D | E | F |
|---|---|---|---|---|---|
| **Reagent** | **Stock conc.** | **Final conc.** | **1 mL** | **4 mL** | **12 mL** |
| Nuclease free water | n/a | n/a | 0.927 mL | 3.708 mL | 11.124 mL |
| CaCl2 | 1 M | 5 mM | 5 uL | 20 uL | 60 uL |
| MgOAC | 1 M | 3 mM | 3 uL | 12 uL | 36 uL |
| Tris-HCl, pH 8.0 | 1 M | 10 mM | 10 uL | 40 uL | 120 uL |
| EDTA | 500 mM | 2 mM | 4 uL | 16 uL | 48 uL |
| DTT | 100 mM | 0.6 mM | 6 uL | 24 uL | 72 uL |
| Protease Inhibitor | 25X | 1X | 40 uL | 160 uL | 480 uL |
| Enzymatics RNase In | 40 U/uL | 0.2 U/uL | 5 uL | 20 uL | 60 uL |

**MACS Buffer. Make a stock without Protease In and add when using.**

| A | B | C | D | E | F |
|---|---|---|---|---|---|
| **Reagent** | **Stock conc.** | **Final conc.** | **1 mL** | **5 mL** | **10 mL** |
| PBS | 1X | 1X | 0.93 mL | 4.65 mL | 9.3 mL |
| Superase In | 20 U/uL | 0.05 U/uL | 2.5 uL | 12.5 uL | 25 uL |
| Enzymatics RNase In | 40 U/uL | 0.05 U/uL | 1.25 uL | 6.75 uL | 12.5 uL |

**PBS + Rnase In. Make fresh for each use.**

1 Section flash-frozen skeletal muscle into aliquots according to desired nuclei yield and store on dry ice or at **-80 °C** .

Expect yield of 2000-4000 nuclei per mg of skeletal muscle tissue.

2 Prepare buffers and chill components:

| A | B | C |
|---|---|---|
| **Tissue mass** | **MACS buffer** | **NPB buffer** |
| 10-50mg | 1 mL | 2 mL |
| 50-100mg | 2 mL | 4 mL |
| >100mg | 3 mL | 1 mL per 25 mg |



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Note: May require further optimization.

- Prepare buffer fresh on day of nuclei isolation
- Precool centrifuge to **4 °C**
- Precool all buffers at **4 °C**
- Place gentleMACS dissociator in cold room / chiller
- Chill gentleMACS M tubes on ice
- Chill 5.0 mL Eppendorf tubes on ice

3 Transfer sectioned frozen tissue to gentleMACS M tubes.

4 Add the recommended volume of MACS buffer to gentleMACS M tube.

Allow tissue to thaw in MACS buffer for approximately 60 seconds on ice.

5 Homogenize with Miltenyi tissue dissociation protocol: "Protein_01_01" on the gentleMACS instrument in the chiller/cold room.

6 Briefly centrifuge the gentleMACS M tube to pull all the homogenate to the bottom. 15s
**160 x g, 4°C, 00:00:15**

7 Filter homogenate through 30 uM CellTrics filter into 5 mL Eppendorf Tubes.

8 Wash gentleMACS M tube with another 1 mL of MACS buffer and filter the wash.

9 10m
**900 x g, 4°C, 00:10:00**

Use ramp rate: 3/9 acceleration and 3/9 deceleration.



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10 Decant and discard the supernatant.

11 Resuspend the pellet with the recommended NPB buffer.

12 Gently rotate the sample in cold room/chiller for **00:10:00** . 10m

Users should optimize lysis timing for different samples. (i.e., 5 minutes for cardiac tissues).

13 Centrifuge permeabilized nuclei at **900 x g, 4°C, 00:10:00** . 10m

14 Decant and discard the supernatant.

15 Resuspend the pellet in 500 uL PBS + RNase In.

For SNARE-seq2: immediately fix nuclei by adding (1:1) 500 uL PBS + Rnase In + 1% paraformaldehyde. (Make 1 mL : 937.5 uL PBS+RI + 62.5 uL 16% methanol-free PFA). Incubate on ice for **00:10:00**

16 **QA/QC**: Count the nuclei. Mix 1:1 volume of cellular suspension with a staining solution (ie DAPI). Load 10 uL of mixture onto a Biorad Cell Counter slide then count with a BioRad TC20 Cell Counter. Gate 4 uM-6 uM for nuclei sizes.

**QA/QC**: Check nuclei integrity under fluorescent microscope using DAPI channel. Nuclei



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17 should appear distinct, have rounded borders and the majority occurring as singlets.

If high clumping is observed: bring total volume to ~1 mL with PBS+0.1%BSA and filter the sample through a 30 uM CellTrics filter. Pellet **900 x g, 4°C, 00:10:00** . Then resuspend again in 100 uL PBS + Rnase In.

If high debris is observed (low DAPI+): bring volume to ~ 1 mL with PBS+0.1%BSA and pellet: **900 x g, 4°C, 00:10:00** . Then resuspend again in 100 uL PBS + RNase In.



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