ABSTRACT

This protocol is for nuclear extraction from human heart tissue for single cell sequencing.

MATERIALS

Required Solutions and Reagents

- **DAPI**
- **Methanol (100 %)**

**Stock cell lysis buffer** (store at 4 °C): 10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl₂

Recipe for 1 ml **Cell lysis buffer** - *prepare fresh* - 10 ml/sample required

- 950 µL stock cell lysis Buffer
- 10 µL IGEPAL CA-630
- 10 µL 20 U/µl SUPERase•In RNase Inhibitor
Keywords: nuclear extraction, human heart tissue, heart tissue, single cell sequencing

(A) OptiPrep (product stock)

(B) OptiPrep diluent (store at 4 °C): 150 mM KCl, 30 mM MgCl₂, 120 mM Tris-HCl (pH7.4)

(C) Working solution - prepare fresh - 50 % iodixanol - 13.5 ml/sample required

- 11.25 mL Optiprep (A)
- 2.25 mL Optiprep diluent (B)
- 135 µL 20 U/µl SUPERase•In RNase Inhibitor
- 135 µL 10 % BSA
- 135 µL 0.2 M Spermine

Stock homogenization buffer: 0.25 M Sucrose, 25 mM KCl, 5 mM MgCl₂, 20 mM Tris-HCl

(D) Homogenization buffer - prepare fresh - 6 ml/sample required

- 970 µL stock homogenization buffer
- 10 µL 20 U/µl SUPERase•In RNase Inhibitor
- 10 µL 10 % BSA
- 10 µL 0.2 M Spermine

Recipe for 1 ml of Nuclear buffer - prepare fresh - 4 ml/sample required

- 940 µL stock homogenization buffer
- 10 µL 20 U/µl SUPERase•In RNase Inhibitor
- 10 µL 10 % BSA
- 10 µL 0.2 M Spermine
- 10 µL 10 % Tween-20
Gradient Solutions

<table>
<thead>
<tr>
<th></th>
<th>Working Solution (C) / ml</th>
<th>Homogenization buffer (C) / ml</th>
<th>per sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 % Optiprep</td>
<td>1</td>
<td>0.6</td>
<td>1.6</td>
</tr>
<tr>
<td>35 % Optiprep</td>
<td>7</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>40 % Optiprep</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>per solution</td>
<td>12</td>
<td>4.6</td>
<td></td>
</tr>
</tbody>
</table>

SAFETY WARNINGS

⚠ Please see SDS (Safety Data Sheet) for hazards and safety warnings.

BEFORE START INSTRUCTIONS

Note: Be organized, diligent and keep sample and solutions cold at all times

Prepare required solutions and buffers fresh.

On dry ice

1. Put on dry ice:
   - flat bottom mortar and pestle, hammer and forceps
   - sample-flash frozen heart tissue
   - scale plate

Once everything is cold

2. Assemble scale and cover plate with weighing paper.

3. Weigh 300 mg tissue.
4 Transfer tissue *immediately* into mortar and cover with pestle.

5 Pulverize tissue in mortar using pestle and hammer.

6 Hammer gently, scrape off tissue stuck to pistill.

7 Hammer *again 3-6x*.

**In laminar air hood - on dry ice**

8 Transfer pulversized tissue in 6 cm dish containing 4 mL cell lysis buffer *on ice*.

9 Start timer.

10 Segregate particles and transfer into *douncer A* with transfer pipette.
11 Wash plate with 2 mL cell lysis buffer and transfer into douncer A.

12 Dounce carefully 30x.

13 Filter through 100 µm mesh in 50 ml Falcon tube.

14 Wash douncer A with 2 mL cell lysis buffer and filter as well.

15 Keep 10 µL for QC #1.

16 Transfer into douncer B.

17 Dounce 20x.

18 Filter through 40 µm mesh in 50 ml Falcon tube.
19 Wash *douncer* B with \( 2 \text{ mL cell lysis buffer} \) and filter as well.

20 Transfer into 15 ml Falcon tube.

21 Take time: should take \( 00:10:00 \).

22 Spin \( 400 \times g, 4\,^\circ C, 00:07:00 \).

23 Aspirate supernatant.

### Centrifugation

24 Note

During testing, collect all 3 phases of Optiprep centrifugation, add same volume of nuclear buffer and spin to check for quality and quantity of separation of nuclei and cell debris. Adjustments may be required.

Resuspend pellet in \( 600 \mu L \) homogenization buffer (D).

25 Add \( 1 \text{ mL Optiprep working solution and mix carefully (C)} - 30 \% \) iodixanol.
26  Keep 10 µL for QC #2.

27  Transfer into centrifugation tube (40ml).

28  Underlayer carefully nuclear sample with 8 mL 35 % iodixanol using serological pipette.

29  Underlayer carefully both layers with 4 mL 40 % iodixanol.

30  Centrifuge at 8,000 x g, 4°C, 00:20:00; no breaks.

31  Collect ring of nuclei at 35 % - 40 % iodixanol interface.

32  Add same volume of nuclear buffer.

33  Spin at 500 x g, 4°C, 00:10:00.
34 Aspirate carefully and resuspend in nuclear buffer.

35 Stain 5 µL of sample as well as all fractions of QC with DAPI.

36 Check nuclei for complete lysis, nuclei morphology, purity and count.

37 Resuspend nuclei in 100 µL nuclear buffer.

38 Add drop wise 400 µL 100 % methanol to suspension and transfer into -80 °C.