

Nuclear Extraction from Human Heart Tissue

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Protocol for nuclear extraction from human heart tissue for single cell sequencing

In 1 collection

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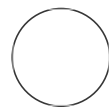
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Protocol status: Working
 We use this protocol and it's working

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 33104



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ABSTRACT

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

ATTACHMENTS

[nuclei_isolation-human_heart-CP-0220.pdf](#)

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

Keywords: nuclear extraction, human heart tissue, heart tissue, single cell sequencing

- 10 μ L IGEPAL CA-630
- 10 μ L 20 U/ μ l SUPERase•In RNase Inhibitor
- 10 μ L 10 % BSA
- 10 μ L 0.2 M Spermine
- 10 μ L 10 % Tween-20

(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 μ L 20 U/ μ l SUPERase•In RNase Inhibitor
- 135 μ L 10 % BSA
- 135 μ L 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 μ L stock homogenization buffer
- 10 μ L 20 U/ μ l SUPERase In RNase Inhibitor

- 10 μ L 10 % BSA
- 10 μ L 0.2 M Spermine

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

- 940 μ L stock homogenization buffer
- 10 μ L 20 U/ μ l SUPERase \cdot In RNase Inhibitor
- 10 μ L 10 % BSA
- 10 μ L 0.2 M Spermine
- 10 μ L 10 % Tween-20

Gradient Solutions

	Working Solution (C) / ml	Homogenization buffer (C) / ml	per sample
30 % Optiprep	1	0.6	1.6
35 % Optiprep	7	3	10
40 % Optiprep	4	1	5
per solution	12	4.6	

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.

In laminar air hood - on dry ice

- 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 wet ice

- 8 Transfer pulverized 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 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 x g, 4°C, 00:07:00 .
- 23 Aspirate supernatant.



Centrifugation

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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 μ L homogenization buffer (D).


25 Add  1 mL Optiprep working solution and mix carefully (C)  - 30 % iodixanol.

26 Keep  10 μ L 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.

Fixation

37 Resuspend nuclei in  100 µL nuclear
buffer

38 Add drop wise  400 µL  100 % ( -20 °C) **methanol** to suspension and transfer into
 -80 °C

