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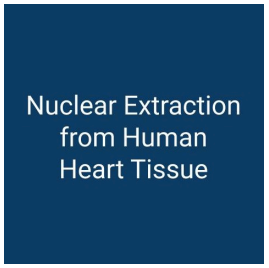
Protocol for nuclear extraction from human heart tissue for single cell sequencing



In 1 collection

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Human Cell Atlas Metho...

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

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

Abstract

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

Attachments

PDF

nuclei_isolation-hum...

59KB

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 $MgCl_2$

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
- 10 µL 10 % BSA
- 10 µL 0.2 M Spermine
- 10 µL 10 % Tween-20

(A) OptiPrep (product stock)

(B) OptiPrep diluent (store at 4 °C): 150 mM KCl, 30 mM $MgCl_2$, 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 $MgCl_2$, 20 mM Tris-HCl

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

- 970 µL stock homogenization buffer
- 10 µL 20 Uµl 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

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

Safety warnings

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

Before start

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 foreceps
- 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 3-6*x.

In laminar air hood – on wet 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 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

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 seperation of nuclei and cell debris. Adjustments may be required.

Resupend pellet in 600 µL homogenization buffer (D) .

25 Add 1 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 [M] 35 % iodixanol using serological pipette.

29 Underlayer carefully both layers with 4 mL [M] 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 nucelar buffer .

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