Mar 07, 2020

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

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1 Works for me dx.doi.org/10.17504/protocols.io.bcjqiumw

Human Cell Atlas Method Development Community

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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 TEXT

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 STARTING

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

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** .