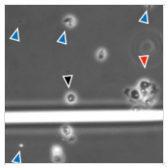

Nuclei isolation from human kidney for single-nucleus RNA-seq

Ben Humphreys[1], Yuhei Kirita[2]

[1]Washington University in St. Louis, [2]The Humphreys Lab

dx.doi.org/10.17504/protocols.io.nahdab6

Oct 05, 2018

Working

Human Cell Atlas Method Development Community

Ben Humphreys
Washington University in St. L...

ABSTRACT

This protocol is based upon *Habib, N et al., Nature Methods 14: 955-958, 2017* and Basu et al., Protocol Exchange 2017: DroNc-seq step-by-step with adaptions for adult human kidney including: Tissue mincing, homogenization strokes, addition of protease inhibitor and RNasin and adjustments to strainer size and sequence.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

This protocol is based upon *Habib, N et al., Nature Methods 14: 955-958, 2017* and *Basu et al., Protocol Exchange 2017: DroNc-seq step-by-step*

Nuclei isolation protocol.pdf

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

All steps are performed on ice or in cold room (4°C) to minimize RNA degradation.

Storage temperatures

Reagent	Storage
1x DPBS	4°C
Nuclei EZ Lysis Buffer	4°C
cOmplete ULTRA Tablets, Mini, EDTA-free, EASYpack	4°C
RNasin Plus Ribonuclease Inhibitors	-20°C
SUPERaseIN RNase Inhibitor	-20°C
RNase free H_2O	RT
RNaseZap	RT

MATERIALS

NAME	CATALOG #	VENDOR
1x DPBS	14190144	Gibco - Thermo Fischer
Nuclei EZ Lysis Buffer	N-3408	Sigma

NAME	CATALOG #	VENDOR
cOmplete ULTRA Tablets, Mini, EDTA-free, EASYpack	05 892 791 001	Roche
RNasin Plus Ribonuclease Inhibitors	N2615	Promega
SUPERaseIN RNase Inhibitor	AM2696	Thermo Fisher Scientific
RNase free H2O	AM9938	Thermo Scientific
RNaseZap	AM9780	Ambion
KONTES Dounce Tissue Grinders	KT885300-0002	Kimble Chase
pluriStrainer 200 µm	43-50200	pluriSelect
pluriStrainer 40 µm	43-50040	pluriSelect
pluriStrainer 5 µm	43-50005	pluriSelect
Fuchs-Rosenthal disposable hemocytometer	DHC-F015	INCYTO
Falcon 15 mL Polystyrene Conical Tube	352095	Fisher Scientific
RNase-free 50 ml Conical Tubes	AM12502	Ambion
TPP 60mm Tissue Culture Dishes	TP93060	MIDSCI

BEFORE STARTING

Buffers and Solutions

Nuclei lysis buffer 0 (NLB0) working solution
- 10 ml of Nuclei EZ Lysis Buffer
- 1 tablet of cOmplete ULTRA tablets

Nuclei lysis buffer 1 (NLB1) working solution - make 4 ml per < 8 mm^3 tissue
- 4 ml of NLB0
- 20 µl of RNasin Plus
- 20 µl of SUPERaseIN

Nuclei lysis buffer 2 (NLB2) working solution - make 4 ml per < 8 mm^3 tissue
- 4 ml of Nuclei EZ Lysis Buffer
- 4 µl of RNasin Plus
- 4 µl of SUPERaseIN

Nuclei suspension buffer (NSB)
- 2 ml of DPBS
- 2 µl of RNasin Plus

1 Precool all instruments and buffers.

> NOTE
> All steps are performed on ice or in cold room (4°C) to minimize RNA degradation.

2 Start with kidney cubes (either fresh or snap frozen, smaller than 8 mm^3) and place on a 60 mm dish.

3 Add 1 ml of NLB1, then mince very well with a fresh razor blade.
1 ml NLB1

4 Transfer the minced tissue with NLB1 into a Dounce tissue grinder and add another 1 ml of NLB1.
1 ml NLB1

5 Grind 20-30 times with a loose pestle, then pass the homogenate through a 200 µm strainer and collect in a 50 ml conical tube.

NOTE
To reduce heat caused by friction, homogenization should be performed gently on ice.

6 Transfer the homogenate again into a Dounce tissue grinder.

7 Grind the homogenate 10-15 times with a tight pestle.

8 Add 2 ml of NLB1 and incubate for 5 min on ice.
2 ml NLB1
00:05:00 Incubation on ice

9 Pass the homogenate through a 40 µm strainer into 50 ml conical tube.

10 Transfer the homogenate into a 15 ml conical tube.

11 Centrifuge the tube at 500 G for 5 min at 4°C.
4 °C Centrifugation
00:05:00 Centrifugation at 500 G

12 Discard supernatant.

13 Carefully suspend the pellet in 4 ml of NLB2.
4 ml NLB2

14 Incubate on ice for 5 min.
00:05:00 Incubation on ice

15 Centrifuge at 500 G for 5 min at 4°C.

NOTE
We use a fixed angle rotor.

4 °C Centrifugation

00:05:00 Centrifugation at 500 G

16 Discard supernatant.

17 Carefully suspend the pellet in 2 ml of NSB.

2 ml NSB

18 Pass the suspension through a 5 µm strainer into 50 ml conical tube.

19 Count nuclei by hemocytometer.

EXPECTED RESULT