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Nuclei prep for single cell RNA/ATAC seq from intestinal surgical samples

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1 Works for me dx.doi.org/10.17504/protocols.io.bmbak2ie

Human Cell Atlas Method Development Community | Helmsley project_Basu lab

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ABSTRACT

Isolating high-quality nuclei from intestinal surgical tissues is critical for single cell RNA/ATAC-seq. This protocol provides details on nuclei preparation for such application, with an overall working time of less than an hour, if FACS sorting is not incorporated.

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PROTOCOL CITATION

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KEYWORDS

intestine, single cell, RNA-seq, ATAC-seq, Surgical

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GUIDELINES

The human intestinal tissue are obtained with patient consent and approval by the Institutional Review Board at the University of Chicago (IRB Number: 15573A). All samples are processed for research use only.

MATERIALS

NAME	CATALOG #	VENDOR
5M Sodium Chloride, 1000ml	V4221	Promega
BSA		Sigma Aldrich
RiboLock RNase Inhibitor (40 U/µL)	EO0381	Thermo Fisher
Cell strainer 100 micron	431752	Corning
0.5M EDTA	2482-500	Fisher Scientific

NAME	CATALOG #	VENDOR
10 x PBS no calsium no magnesiusm	BP399500	Fisher Scientific
UltraPure™ DNase/RNase-Free Distilled Water	10977023	Thermofisher
Red blood cell lysis buffer 10x	130-094-183	Miltenyi Biotec
40um Cell Strainer	22363547	Fisher Scientific
Tween 20	P7949	Sigma Aldrich
1M Tris-HCl pH 7.5	15567027	Thermo Fisher Scientific
1M CaCl2	21115	Sigma-aldrich
1M MgCl2	63069	Sigma-aldrich

MATERIALS TEXT

Lysis buffer 10 ml (make fresh)
5 ml 2x ST buffer
300 ul 1% Tween-20
50 ul 2%BSA
10 ul RNAse Inhibitor stock
4.64 ml ultra pure water

2x ST buffer 10 ml (Store at 4 Celsius up to 1 month)
292 mM NaCl
20 mM Tris-HCl pH 7.5
2 mM CaCl2
42 mM MgCl2
Bring up to volume with ultra pure water

RBC lysis buffer 10 ml
1 ml Red blood cell lysis buffer 10x (warmed to room temperature)
9 ml ultra pure water

2% BSA 10 ml (Store at 4 Celsius up to 1 month)
0.2 g BSA
10 ml ultra pure water

1% Tween-20 10 ml (Store up to 1 month)
1ml 10% Tween-20
9 ml ultra pure water

Nuclei suspension buffer 10 ml (make fresh)
10 ul RNAse Inhibitor stock
50 ul 2% BSA
9.94 ml 1x PBS

1x PBS 500 ml (filter through 0.2 uM filter top)
50 ml 10x PBS
450 ml ultra pure water

DISCLAIMER:

The lysis buffer is formulated from the recipe in:
Drokhlyansky E, Smillie CS, Van Wittenberghe N, et al. The Human and Mouse Enteric Nervous System at Single-Cell Resolution [published online ahead of print, 2020 Aug 21].Cell. 2020;S0092-8674(20)30994-6. doi:10.1016/j.cell.2020.08.003

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BEFORE STARTING

Record the wait time between tissue removal from the patient and arrival at the lab. We don't recommend processing tissue older than 3 hours since the RNA will degrade.

Pre-processing of surgical samples

1 Rinse surgical samples in ice-cold PBS for three times.
00:02:00

2 Place the surgical samples in a 100 mm petri dish with cold HBSS, and isolate mucosa layer by Iris scissors.
00:03:00

3 Weigh mucosa layer and cut the tissue into small pieces (<200 mg, 8mm x 8mm) on ice.
00:05:00

4 Place each small piece in a 1.7 ml Eppendorf tube on ice.

Flash freeze the unused tissus in liquid nitrogen and store those tissues in liquid nitrogen/at - 80 Celsius.

Lyse surgical tissues for nuclei

5 Mince the tissue (fresh or frozen) in 0.5 ml lysis buffer by Iris Scissors on ice for 5 minutes.
00:05:00 **On ice**

6 Transfer the minced tissue in lysis buffer to a 5 ml conical tube. Add an additional 3 ml lysis buffer to the tube.

7 Mix the tissue with lysis buffer by inverting the tube and incubate on ice for 10 minutes.
00:10:00 **On ice**

Agitate tissue in lysis buffer by inverting the tube every 2 minutes.

Enrich nuclei from the lysate

8 Wet a 100 micron cell strainer with 1 ml lysis buffer.

9 Filter the lysate through the strainer on ice and wash the strainer with 3 ml NSB. Keep the flow through as this is where your nuclei are.

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00:05:00 On ice

If FACS sorting is incorporated after nuclei enrichment, NSB in this experiment is supplemented with Hoechst 33342 10 ug/ml and WGA-Texas Red 1 ug/ml.

10 Wet a 40 micron cell strainer with 1 ml lysis buffer.

11 Filter the flow-through on this strainer on ice and wash the strainer by 3 ml NSB. Keep the flow through as this is where your nuclei are.
00:03:00 On ice

12 Spin the final flow-through in a 15 ml conical tube, 500 g x 5 minutes at 4 Celsius.
500 x g, 4°C, 00:05:00

13 For fresh tissues, suspend the pelleted nuclei in 1 ml NSB with gentle pipetting and continue with step 14.
For frozen tissues, suspend the pelleted nuclei in 200 ul NSB with gentle pipetting and continue with step 18.

14 Add RBC lysis buffer 10 ml to the fresh tissue nuclei suspension.

15 Invert the tube three times to mix and incubate at room temperature for 2 minutes.
00:02:00

The incubation may be extended (up to 10 minutes) to fully lyse RBCs.

16 Spin the nuclei suspension in a 15 ml conical tube, at 600 g x 5 minutes at 4 Celsius.
500 x g, 4°C, 00:05:00

17 Suspend pelleted nuclei in 200 ul NSB or other nuclei suspension buffer as recommended by the single cell platform. Use gentle pipetting to resuspend as the nuclei are very fragile.

Prepare nuclei for down stream applications

18 Stain the nuclei with Hoechst 33342 10 ug/ml and WGA-Texas Red 1 ug/ml. Count the nuclei.

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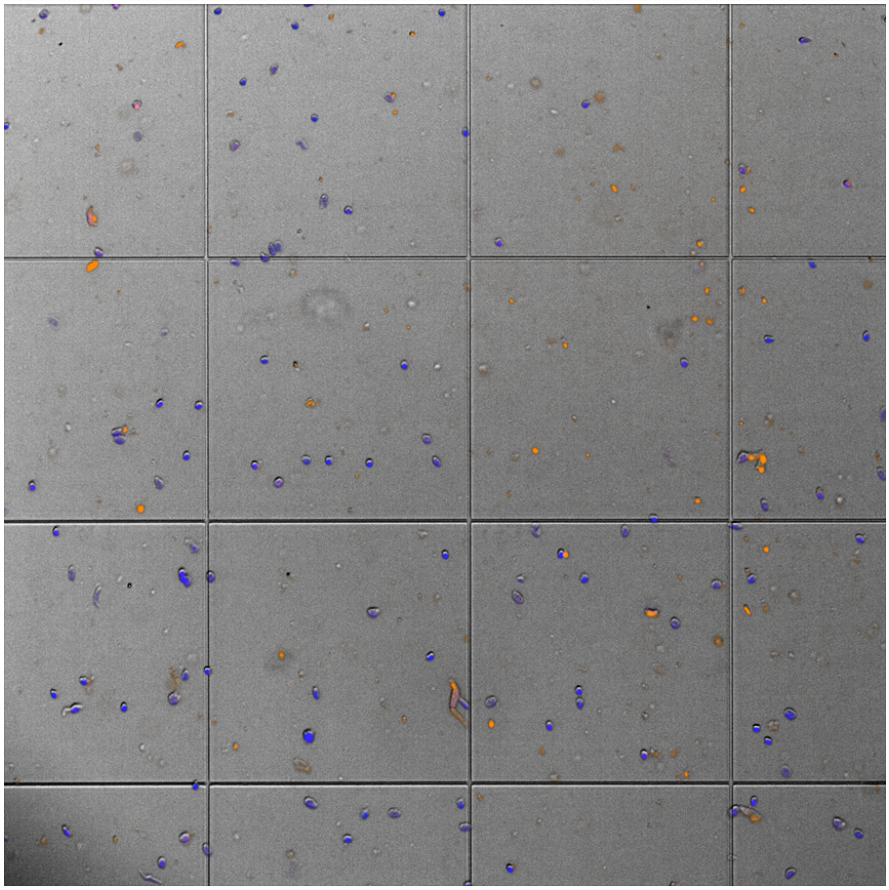
00:05:00

Skip this staining step for nuclei prepared for sorting.



Objective lens 10x_ Nuclei are stained by Hoechst 33342-blue. Nuclei with better integrity are co-stained by WGA-orange.

19 Adjust the nuclei concentration to the desired concentration using nuclei suspension buffer.

20 Proceed with downstream applications.

After FACS sorting, nuclei are spun down at 700 g x 5 minutes at 4 celsius and stained by Hoechst 33324 10 ug/ml. Count the nuclei again and adjust the nuclei concentration accordingly.

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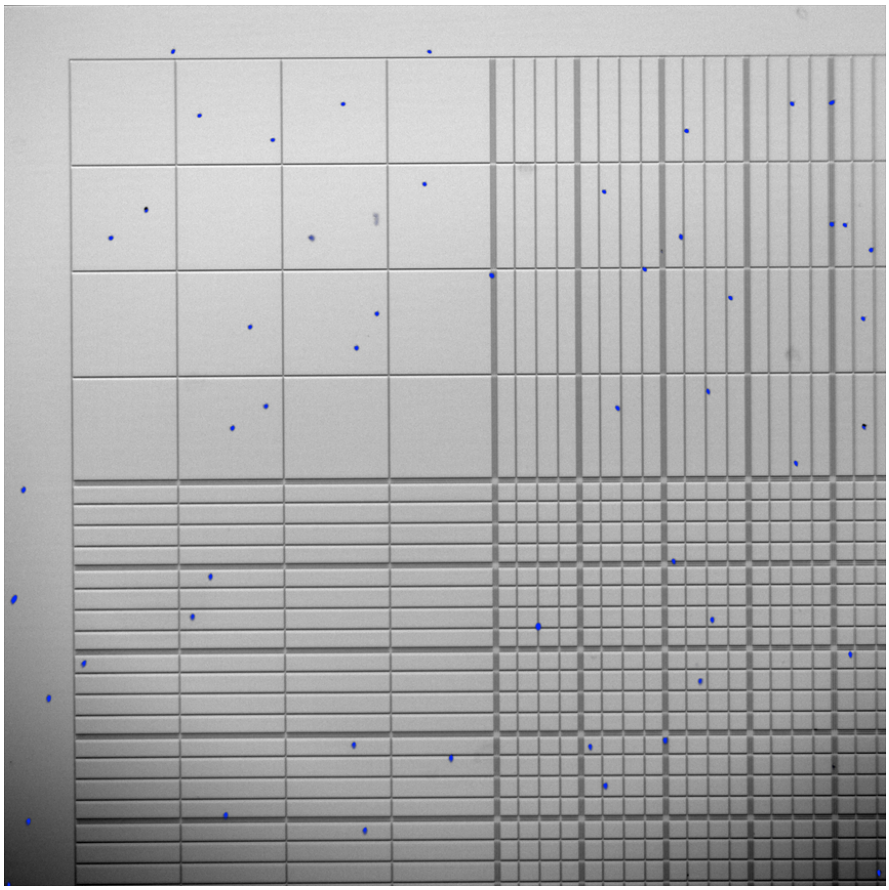

Objective lens 4x _Count sorted nuclei with Hoechst 33324 staining.

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