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Nuclei isolation from snap frozen human pancreatic tissue using a citric acid buffer

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

Human Cell Atlas Method Development Community

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ABSTRACT

A protocol to isolate nuclei from snap-frozen pancreatic tissue and protect RNA from RNAse-mediated degradation

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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single-cell RNA-seq, sNuc-RNA-seq, citric acid buffer, snATAC-seq

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MATERIALS

NAME	CATALOG #	VENDOR
DTT	D0632	Sigma Aldrich
citric acid monohydrate	33114	Sigma-aldrich
Sucrose	S7903	Sigma Aldrich

NAME	CATALOG #	VENDOR
Dounce homogenizers	D8938-1SET	Sigma
RNase Inhibitor (40 U/µL)	634888	Takara
Falcon® 5 mL Round Bottom Polystyrene Test Tube, with Cell Strainer Snap Cap	352235	Corning
Countess™ Cell Counting Chamber Slides	C10314	
1M MgCl2	AM9530G	Ambion
Nuclease-Free Water	AM9939	Thermo Fisher Scientific
SUPERase• In™ RNase Inhibitor (20 U/µL)	cat# AM2694	Thermo Fisher Scientific
KCl 2M	AM9640G	
Tris buffer solution pH 75 (1 mol/l) Ultrapure Grade		VWR International
Hoechst 33258	H3569	Thermo Fisher Scientific
Countess II Automated Cell Counter	AMQAX1000	Thermo Fisher Scientific

BEFORE STARTING

- **Cool down table-top centrifuge to 4°C.**
- **Place douncers and pestles on ice/in a fridge to cool them down 0-4°C.**
- **Prepare the following buffers and keep them on ice:**

S25

Reagent	Amount (µL)
Sucrose 1.5M	500.00
Citric Acid 250mM	300.00
Hoechst	3.00
Nuclease-free H2O	2197.00
TOTAL	3000.00

S88

Reagent	Amount (µL)
Sucrose 1.5M	1000.00
Citric Acid 250mM	170.00
Nuclease-free H2O	530.00
TOTAL	1700.00

Resuspension Buffer

Reagent	Amount (µL)
KCl 2M	12.50
MgCl2 1M	3.00
Tris-buffer pH=7.5 1M	50.00
RNaseIn 40U/µL	10.00
DTT (1M)	1.00
SuperaseIn 20U/µL	20.00
Hoechst	1.00
Nuclease-free H2O	902.50
TOTAL	1,000.00

1 Add 1 mL of cold S25 buffer to the douncer placed on ice

1 mL S25 buffer

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2 Transfer the tissue in the douncer, crush it with one stroke of the loose pestle and dislodge the tissue from the bottom using a P200 tip if necessary

3 Incubate the tissue for 5 min on ice

4 °C on ice

00:05:00 incubation

4 Crush the tissue with 5 more strokes of the loose pestle and leave to incubate for 5 minutes

4 °C on ice

00:05:00 incubation

5 Homogenize the tissue with 3 more strokes of the loose pestle and 5 more strokes with the tight pestle

6 Filter through a FACS-tube with cell strainer cap (35 µm)

7 Wash filter with 250 µL of S25 buffer

250 µl S25 buffer

8 Transfer the nuclei suspension to a 1.5 mL eppendorf tube and centrifuge at 4°C, 500g for 5 minutes

00:05:00 centrifugation

You will probably not see the pellet, but nuclei are on the wall of the tube! Be careful when aspirating, leave a few µL of liquid at the bottom in the next step!

9 Resuspend the nuclei in 1 mL of S25 buffer and repeat the centrifugation step as in step 8.

1 mL S25 buffer

00:05:00 centrifugation

10 Carefully remove the supernatant, resuspend in 300 µL S25 buffer and load 10 µL of nuclei suspension in a Countess™ Cell Counting Chamber Slide. Count the Hoechst-positive nuclei. Use the brightfield to evaluate the amount of debris present in the suspension and the overall integrity of the nuclei. For an example of Countess images, please see the expected results in Step 13.

300 µl S25 buffer

Step 10 includes a Step case.

High amount of debris

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step case

High amount of debris

If you can see high amount of debris (small fragments in the brightfield view, Hoechst-negative), you can perform a density centrifugation step as described here:

1. Go at the bottom of the nuclei suspension and **gently** add 1 volume (300 µL) of S88 buffer.
2. Centrifuge at 4°C, 1,000g for 10 minutes.
3. Remove the supernatant - **careful not not aspirate nuclei, better to leave a few microliters at the bottom!**
4. Resuspend in 300 µL of S25 buffer and check again on the Countess - you should now have a cleaner suspension!
5. Proceed to **Step 10**

300 µl S88 buffer

300 µl S25 buffer

00:10:00 centrifugation

go here 10

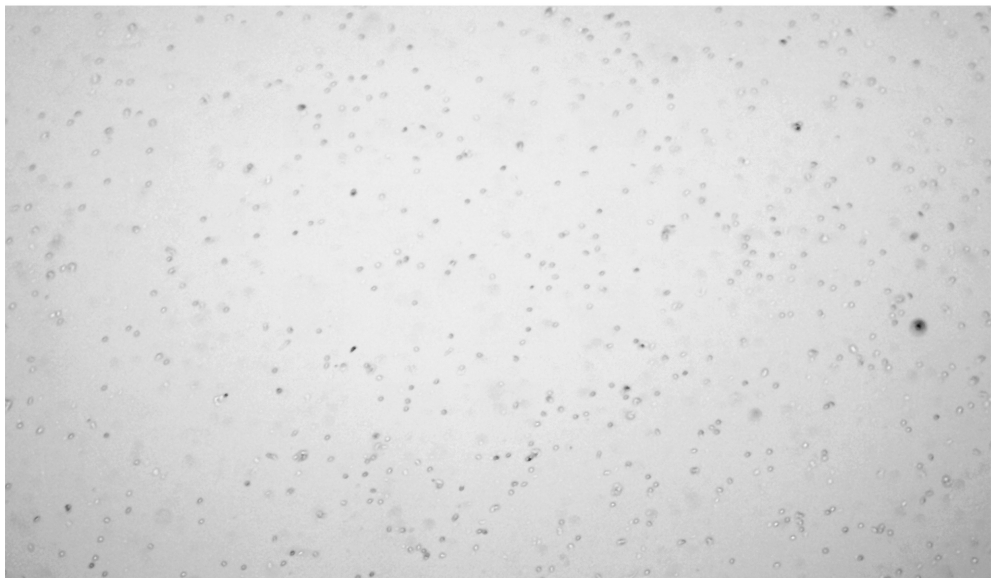
11 Centrifuge the nuclei at 4°C, 500g for 5 minutes

00:05:00 centrifugation

12 Resuspend in appropriate volume of Resuspension Buffer. The volume can be determined based on the desired final concentration; 20% of the nuclei might be lost during the previous centrifugation step.

13 Count nuclei and proceed with FACS sorting of the nuclei or directly to the encapsulation in oil droplets.

14



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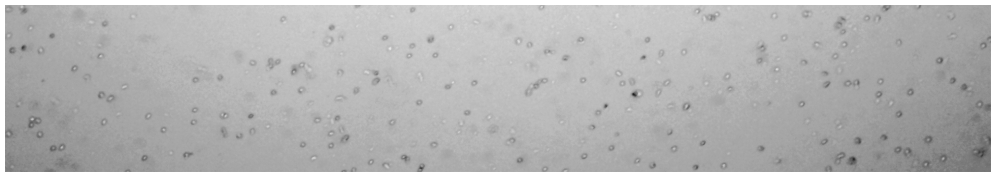
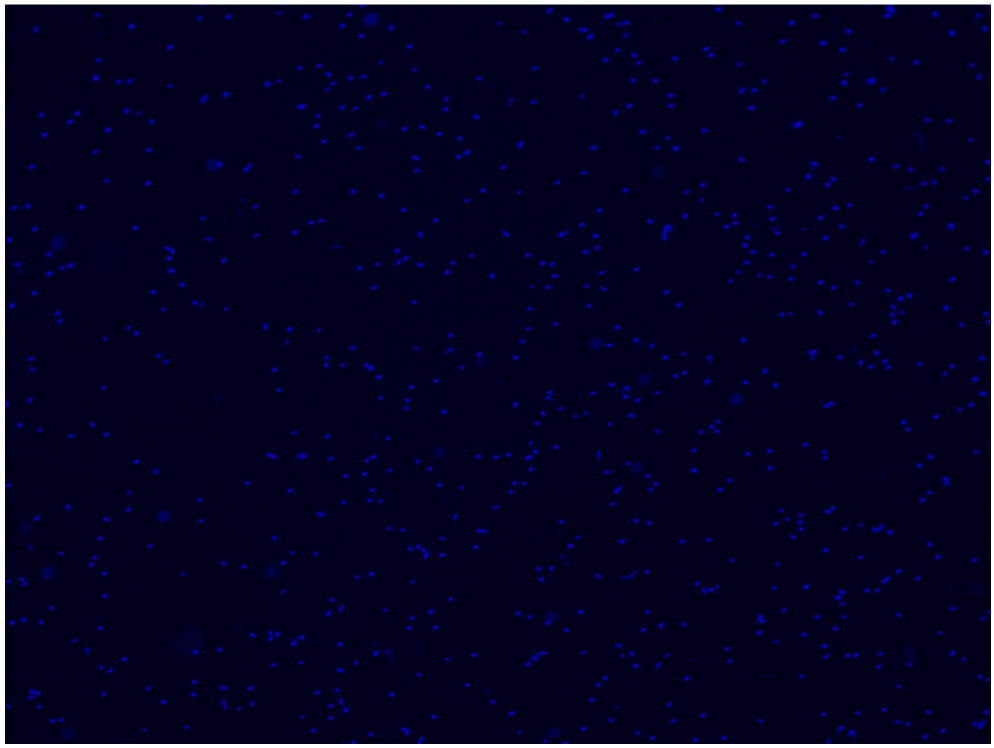


B



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C

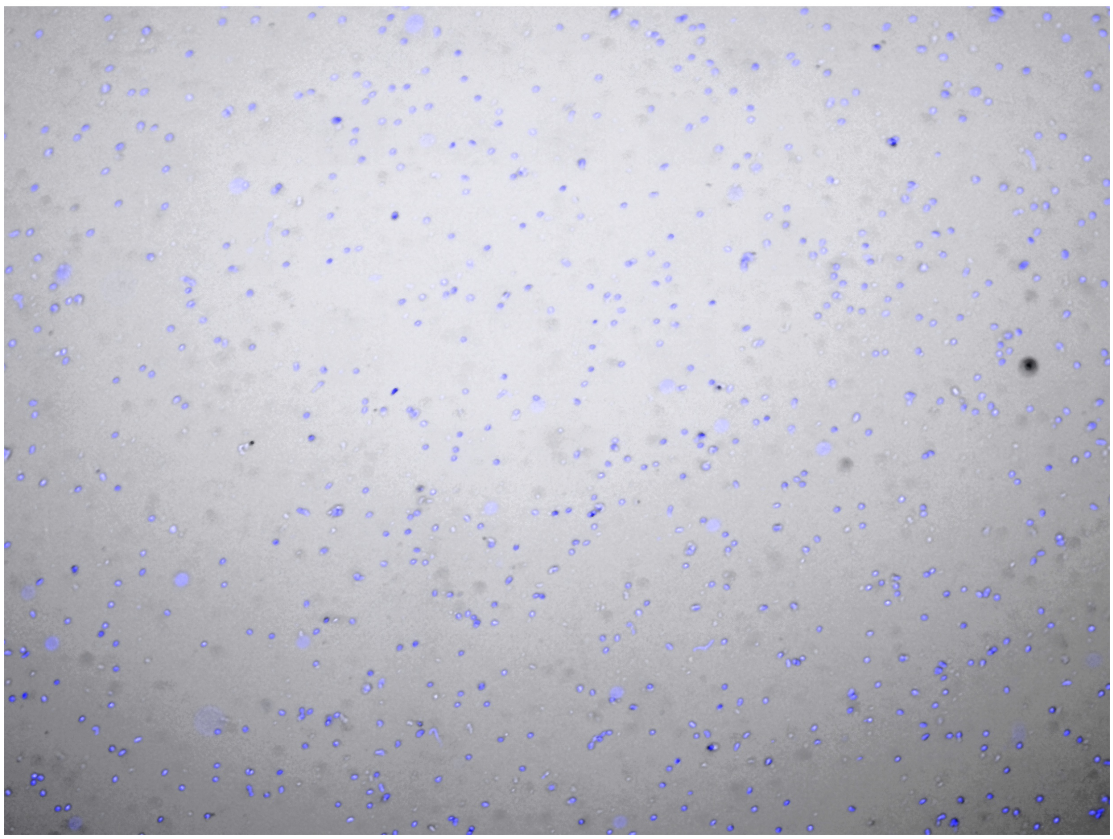

Fig. 1: Representative images of bright-field (A), DAPI (B) and merged (C) fields of human pancreas nuclei isolated with the citric acid protocol. Notably, clumps of nuclei are almost completely absent when using this protocol.

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