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Citric acid nuclei isolation from frozen oesophageal tissue

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1 Works for me dx.doi.org/10.17504/protocols.io.btm6nk9e

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ABSTRACT

Citric acid-based method of isolating nuclei from frozen oesophageal tissue. Protocol adapted from Tosti et al. [1,2] with additional optimisation for oesophageal tissue.

1. Tosti, L., Hang, Y., Debnath, O., Tiesmeyer, S., Trefzer, T., & Steiger, K. et al. (2021). Single-Nucleus and In Situ RNA–Sequencing Reveal Cell Topographies in the Human Pancreas. Gastroenterology, 160(4), 1330-1344.e11. doi: 10.1053/j.gastro.2020.11.010

2. Luca Tosti, Christian Conrad 2020. Nuclei isolation from snap frozen human pancreatic tissue using a citric acid buffer. **protocols.io**https://dx.doi.org/10.17504/protocols.io.tpxempn

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MATERIALS TEXT

MgCl (1M stock)
KCl (1M stock)
Sucrose (1M stock)
NxGen RNAse inhibitor (Lucigen 40U/µL)
Nuclease free water
Citric acid
DTT (1M stock)
SUPERase In RNAse inhibitor (20U/µL)
RNA Later ICE
RNaseZap
1M Tris pH 7.0
PBS

Nuclei lysis buffer:

A	B	C	D
Reagent	Stock concentration	Final concentration	Volume required (µL)
Sucrose	1M	0.25M	1000
Citric acid	1M	25mM	100
Nuclease free H2O			2900

Provides 4ml of nuclei lysis buffer, sufficient to process 1 sample.

Nuclei resuspension buffer:

A	B	C	D
Reagent	Stock concentration	Final concentration	Volume required (µL)
KCl	1M	25mM	25
MgCl2	1M	3mM	3
Tris-buffer	1M	50mM	50
NxGen RNase inhibitor	40U/µL	0.4U/µL	10
DTT	1M	1mM	1
SUPERase In	20U/µL	0.4U/µL	20
Nuclease free H2O			891

Provides 1ml of nuclei resuspension buffer, sufficient to process 1 sample

BEFORE STARTING

1. RNALater-ICE pre-treatment of frozen tissue (**Important**: perform at least one day before nuclei isolation):
(A) Pre-chill a minimum of 10 volumes (compared to the sample mass/ volume) of RNAlater-ICE at 70 or -80°C in a polypropylene tube.
(B) Place frozen tissue (from LN2 or -80°C), no larger than 0.5 cm in the smallest dimension, into the RNAlater-ICE. It is important that the RNAlater-ICE stays cold and the tissue remains frozen, so work quickly. Set up the tubes of RNAlater-ICE and tissue samples in a container of dry ice. For the frozen tissue, simply pipet at least 10 volumes of pre-chilled RNAlater-ICE into the tube. Tightly cap the tube and invert several times to mix.

Soak the tissue in RNAlater-ICE at -20°C for at least 16 hours. Samples transitioned to -20°C in RNAlater-ICE should be left in the RNAlater-ICE and maintain at -20°C or colder.

2. Prepare nuclei lysis and resuspension buffers
3. Pre-chill all reagents, dounce and centrifuge to 4°C

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1 Weigh tissue. 30-40mg of tissue should be used per experiment. If not using the entire sample, quickly remove the tissue required by chopping sample in a petri dish chilled on dry ice (For RNA Later Ice samples see 1.1) with a cold scalpel. Return unused tissue to LN_2.

1.1 If using tissue stored in RNA Later Ice, carry out steps 1 and 2 on wet ice. Pre-chill a petri dish on dry ice and chop tissue into very small pieces (<0.3cm) using a cold scalpel.

2 Add tissue to pre-chilled dounce homogenizer containing 2ml nuclei lysis buffer.

3 While keeping the dounce on ice, homogenize for 5 strokes using pestle A.

4 Incubate tissue on ice in the dounce for 5 minutes.

5 Homogenize with 5 strokes of pestle A followed by 20 strokes with pestle B. The tissue should be well homogenised and the solution in the dounce should be cloudy with no large tissue peices remaining.

6 Filter the contents of the dounce through a 30µm cell strainer. Use an additional 1ml of nuclei lysis buffer to rinse the dounce. Filter the 1ml lysis buffer through the strainer.

7 Centrifuge the nuclei suspension at 500 x g at 4°C for 5 minutes.

8 Carefully remove and discard the supernatant and resuspend the pellet in 1ml of nuclei lysis buffer.

9 Centrifuge the nuclei suspension at 500 x g, 4°C for 5 minutes. Carefully remove the supernatant and resuspend the pellet in 200µL nuclei resuspension buffer.

10 Remove 10µL of nuclei suspension and stain 1:1 with Dapi. If a high nuclei yield is anticipated, dilute the 10µL of nuclei suspension 1:5 with PBS before staining.

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