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Nuclear Extraction from Tissue for FACS

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We use this protocol and it's working

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Abstract

This protocol details the process for pre-tissue preparation and tissue preparation for nuclear extraction for FACS.

Troubleshooting

Before start

Keep everything On ice.

Pre-Tissue Preparation

1 Coat all tubes (centrifugation, sample and collection tubes) in coating medium (Overnight) ([M] 1 % (v/v) BSA, filtered).

2 Cool ultracentrifuge to (4 °C) prior to centrifugation

3 Pre-weigh the DTT needed.

4 Keep douncers (On ice).

Tissue Preparation

10m

5 Put (2.8 mL sucrose solution) in centrifuge tube.

6 Mix (6 mL sucrose) + (2 mL lysis solution) in 15 ml falcon tube.

7 If the tissue is large (>2 mm3), chop the tissue with a razor.

8 Collect chopped tissue with (0.5 mL lysis buffer) and transfer to douncer. Repeat with another (0.5 mL lysis buffer) to get all tissue.

9 Dounce: 5-7x loose, 5-7x tight.

10 Pour douncer content into falcon tube with sucrose/lysis buffer mixture and flip upside down a few times. Pour (1 mL) of tube content back and forth from douncer to collect remaining nuclei.

11 Carefully layer the (8.5 mL mix of lysis/sucrose/nuclei) to the centrifuge tube, on top of sucrose.

12 Balance the tubes (≤ 5 mg difference).

13 Place tubes in ultracentrifuge, spin at 15500 rpm , 02:15:00, (rotor dependent, use 30.000xg) .

14 Carefully aspirate off supernatant without disturbing the pellet.

> Note
>
> Due to the viscosity, try to aspirate the inside of the tube to get rid of all supernatant.

15 Soften pellet for 00:10:00 in 50 µL medium A . 10m

16 Add 100 µL dilution buffer (containing a nuclei marker, such as Draq7) and filter using 70um pipette tip filters into coated tubes.

17 FACS sort, low flowrate, largest nozzle, cooling system on. The sucrose will cause problems at FACS. Avoid this by adding dilution buffer (with your nuclei marker), 100 µL at the time (usually need 300 µL - 400 µL for dilution to avoid problems – add that even before starting to sort).

18 For single nuclei sequence using 10x: Pre-coat the pipette tips needed to load the 10x machine according to step 1.

19 For RNA extraction: Pellet nuclei at 1300 x g , 4°C, 00:15:00 . Remove supernatant and snap freeze for later RNA extraction (or resuspend in 350 µL RLT including beta-mercapto ethanol directly. Use RNeasy micro columns. For very small volumes, resuspend in RLT without pelleting).