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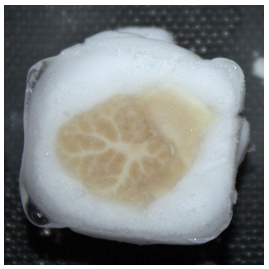
Nuclei Isolation for 10X Multiome Sequencing



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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol is for isolating nuclei for 10X multiome sequencing.

Guidelines

Keep tissue/nuclei on ice as much as possible.

Troubleshooting

Prepare Stock Solutions

1 Make 20 mL **10% BSA** by combining 2 mL of BSA with 18 mL of MilliQ water in a 50 mL falcon tube. (4°C - 2 weeks)

2 Make 20 mL **10% Triton X-100** by combining 18 mL MilliQ water with 2 mL Triton X-100 in a 50 mL tube. Vortex and then incubate at room temperature for 20 minutes. Filter it through a 0.22 µm filter with a syringe into a clean 50 mL tube. (4°C - 1 month)

3 Make 250 mL **Nuclear Isolation Media** by filling a 250 mL bottle with 200 mL of MilliQ water and then adding 2.5 mL 1M Tris, 6.26 mL 1M KCl, 1.25 mL 1M MgCl2, and 21.45 g Sucrose. Shake until sucrose is dissolved then fill to 250 mL with MilliQ water. (4°C - 2 weeks)

Prepare Fresh Solutions

4 Make 3 mL **Homogenization Buffer** per sample by adding 2.895 mL Nuclear Isolation Media (filtered via syringe) to a 5 mL eppendorf. Then add 3 µL 100 mM DTT and 30 µL 10% Triton X-100. Add 15 uL RNAsin and invert to mix. Store on ice.

5 Make 200 uL **Blocking Buffer** per sample by dividing your total desired volume of blocking buffer by 10 to get the amount of 10% BSA in uL. Add this amount to a tube and then fill the remainder with 1X PBS.

Homogenization

6 Clean dounce, scalpel, and forceps using MilliQ water, ethanol, RNase Zap, then MilliQ again. The red-tape forceps are for unfixed tissue.

7 Get tissue sample from -80°C freezer and place on dry ice. Weigh it on a sterile, tared weigh boat.

8 Add tissue to dounce and push it to the bottom using 1 mL of Homegenization Buffer and the pestle. Homogenize the tissue without creating bubbles. Then add the remaining 2 mL of the Homogenization Buffer and continue to dounce until homogenized.

9 Pass all of the nuclei suspension through three FlowMi filters, 1000 µL at a time into a new 5 mL eppendorf.

10 Centrifuge at 900 g/rcf for 10 minutes at 4°C.

11 Discard the supernatant and resuspend the pellet in 200 µL Blocking Buffer. Incubate for 10 minutes on ice.

Cell Count

12 Add 9 uL of sample to a PCR tube and then add 1 uL of acridine orange.
If sample is very concentrated, instead add 2 uL sample to 2 uL of acridine orange and 16 uL 1X PBS.

13 Pipette mix and then add 10 uL to a three-chamber cell counting chip and make note of the channels used (A, B, and/or C).

14 On the cell counter, select Fluorescence Cell Counting → Cell Lines & Primary Cells, Advanced→ Protocol → and then choose "NUCLEI" from the list of protocols. Load the protocol.

If the sample is very concentrated and you are adding 2 uL, select Fluorescence Cell Counting → Cell Lines & Primary Cells→ Protocol → and then choose "NUCLEI" from the protocol list. Load the protocol.

Then go to Settings and choose the appropriate number of channels.

Then hit "Count" and then "Start Count."

15 When the cell count is complete, you will get a reading in cells/mL. Convert this to cells/uL by dividing this number by 1,000.

16 Dilute this nuclei stock to a concentration of 7,000 nuclei/uL.

Example:
(2,000 n/uL)(500 uL) = (7,000 n/uL)(x uL)
x = 2,286 uL
2,286 uL - 498 uL remaining after cell count = 1,788 uL blocking buffer needed to add to sample

17 If there are channels left on the cell counter chip, mark the used channels on the back and place it back in the drawer for future use.

18 Proceed with step 1 of the 10X Multiome protocol.