

Nuclei Isolation with EZ Prep Kit

1. Set the centrifuge temperature to 4°C (Swing bucket centrifuge in the culture room).
2. Prepare nuclei suspension buffer by adding 2 μL of RNase Inhibitor (40U/ μL) per mL of nuclei suspension buffer. Vortex briefly to mix.
3. Keeping everything on ice, add 1 mL EZ PREP buffer (Sigma, NUC101) and tissue piece to the small 2 mL glass tissue grinder. Grind 3 times just to mash the tissue at the bottom of the grinder, then homogenize 25 times with pestle A. Add an additional 1 mL EZ PREP buffer and homogenize 25 times with pestle B. Move slowly to avoid spilling buffer.
4. Use a pipette to transfer to 15 mL conical tube. Add 2 mL EZ PREP buffer to the tissue homogenizer to rinse and pipette into conical tube. Pipette gently to mix.
5. Incubate on ice for 5 minutes.
6. Centrifuge for 5 minutes at 500g and 4°C.
7. Remove supernatant carefully with a pipette. Do not disturb the pellet and leave behind some buffer. Resuspend in 1 mL EZ PREP buffer (pipette gently 20 times up and down), then add 3 mL EZ PREP buffer to wash. Pipette to mix.
8. Incubate on ice for 5 minutes.
9. Centrifuge for 5 minutes at 500g and 4°C.
10. Remove supernatant carefully with a pipette. Do not disturb the pellet and leave behind some buffer. Resuspend in 1 mL nuclei suspension buffer (pipette gently 20 times up and down), then add 3 mL nuclei suspension buffer. Pipette to mix.
11. Centrifuge for 5 minutes at 500g and 4°C.
12. Remove supernatant carefully with a pipette. Resuspend in 500 μL nuclei suspension buffer.
13. In a new labeled Eppendorf tube, make a 1:1 dilution of the nuclei suspension and Trypan Blue. Count nuclei (round blue dots) using haemocytometer slide (C-Chip, DHCN015). Count each 4x4 grid at the corners and average the counts.
14. Concentration of the nuclei: Average count x 2 (dilution in Trypan Blue) x $10^4/\text{mL}$
15. If there are visible clumps of nuclei when counting, re-pipette gently 20 times and then incubate on ice for 5 minutes. Make new 1:1 dilution with Trypan Blue and then recount and check for clumps of nuclei.
16. Fill out the YCGA form and submit samples for 10X loading, or continue with nuclei staining and FACS sorting.