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Nuclei Isolation for 10x Multiome

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

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Abstract

Protocol for isolation of nuclei from cultured human lymphocytes for 10x Multiome processing.

Guidelines

Dead Cell Removal

1. Resuspend cells in 1mL of DCR buffer, pass through 40uM filter and pellet at 300xg for 10min
2. Resuspend each sample in 100uL of DCR buffer
3. Add 5 uL of DCR (Annexin V) Cocktail to each sample
4. Add 5 uL of Biotin Selection Cocktail to each sample
5. Mix and incubate at RT for 3min
6. Vortex RapidSpheres for 30 seconds
7. Add 10 uL RapidSpheres to each sample, mix gently to disperse rapidspheres, add 2.4mL of DCR buffer and mix by gently pipetting up and down 2-3 times
8. Place tubes (without lid) into magnet and incubate at RT for 10 min*
9. Use pipette to transfer supernatant to a new 15 mL tube
10. Add 7.5mL of 2% FBS buffer and pellet at 300xg for 10min at 4C

Nuclei Isolation

1. Resuspend cells in 1mL of PBS + 0.04% PBS and transfer to 2mL tube
2. Pellet at 350xg for 8min at 4C
3. Resuspend in 1mL of PBS + 0.04% PBS and pellet again
4. Remove supernatant and add 100uL of chilled Lysis Buffer, pipette to mix 10x. and incubate on ice for 3min
5. Add 1mL of chilled Wash Buffer to lysed cells and pipette mix 5x
6. Pellet at 500xg for 5min at 4C
7. Remove supernatant and repeat wash two more times for total of 3 washes
8. Resuspend at 1e6 cells/mL in chilled Diluted Nuclei Buffer
9. Count and load cells according to Chromium Single Cell Multiome ATAC + Gene Expression protocol (10x Genomics)

Materials

Dead Cell Removal

- Dead cell removal (DCR) buffer: PBS containing 2% fetal bovine serum (FBS) and 1 mM CaCl
- 2% FBS buffer: PBS containing 2% FBS

Nuclei Isolation

- Diluted Nuclei Buffer: 1X Nuclei Buffer, 1mM DTT, 1U/uL RNase inhibitor
- Wash Buffer: 10mM Tris-HCl, 10mM NaCl, 3mM MgCl2, 1% BSA, 0.1% Tween-20, 1mM DTT, 1U/uL RNase inhibitor
- Lysis Buffer: 10mM Tris-HCl, 10mM NaCl, 3mM MgCl2, 1% BSA, 0.1% Tween-20, 1mM DTT, 1U/uL RNase inhibitor, 0.1% IGEPAL, 0.01% Digitonin

Dead Cell Removal

1 Resuspend cells in 1mL of dead cell removal (DCR) buffer (PBS with 2% FBS and 1mM CaCl), pass through 40uM filter and pellet at 300xg for 10min

2 Resuspend each sample in 100uL of DCR buffer

3 Add 5 uL of DCR (Annexin V) Cocktail to each sample

4 Add 5 uL of Biotin Selection Cocktail to each sample

5 Mix and incubate at RT for 3min

6 Vortex RapidSpheres for 30 seconds

7 Add 10 uL RapidSpheres to each sample, mix gently to disperse rapidspheres, add 2.4mL of DCR buffer and mix by gently pipetting up and down 2-3 times

8 Place tubes (without lid) into magnet and incubate at RT for 10 min*

9 Use pipette to transfer supernatant to a new 15 mL tube

10 Add 7.5mL of 2% FBS buffer and pellet at 300xg for 10min at 4C

Nuclei Isolation

11 Resuspend cells in 1mL of PBS + 0.04% PBS and transfer to 2mL tube

12 Pellet at 350xg for 8min at 4C

13 Resuspend in 1mL of PBS + 0.04% PBS and pellet again

14 Remove supernatant and add 100uL of chilled Lysis Buffer, pipette to mix 10x. and incubate on ice for 3min

15 Add 1mL of chilled Wash Buffer to lysed cells and pipette mix 5x

16 Pellet at 500xg for 5min at 4C

17 Remove supernatant and repeat wash two more times for total of 3 washes

18 Resuspend at 16e6 cells/mL in chilled Diluted Nuclei Buffer

19 Count and load cells according to Chromium Single Cell Multiome ATAC + Gene Expression protocol (10x Genomics)