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Preparation of B cells for scRNAseq

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

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Abstract

This protocol details activated B cell preparation and sample hashtagging for Chromium Single Cell Gene Expression (10x Genomics).

Materials

- EasySep™ Dead Cell Removal (Annexin V) Kit (STEMCell Technologies, Cat. # 17899)
- TotalSeq™-C0262 anti-human Hashtag 12 Antibody (Biolegend, Cat. # 394683)
- TotalSeq™-C0263 anti-human Hashtag 13 Antibody (Biolegend, Cat. # 394683)
- TotalSeq™-C0264 anti-human Hashtag 14 Antibody (Biolegend, Cat. # 394683)
- TotalSeq™-C0265 anti-human Hashtag 15 Antibody (Biolegend, Cat. # 394683)

Dead cell removal

1 Pellet cells at 300xg for 10min

2 Resuspend in 100uL Dead Cell Removal Buffer and filter through 40uM cell strainer

3 Add 5uL of Annexin V Cocktail to sample

4 Add 5uL of Biotin Selection Cocktail to sample, mix and incubate at RT for 3min

5 Vortext RapidSpheres for 30 sec and add 10uL to each sample, flick to mix

6 Add 2.4mL of Dead Cell Removal buffer and mix again

7 Place tubes on magnet and incubate at RT for 10 min

8 Transfer supernatant to new 15mL tube

9 Aliquot

Sample hashtagging

10 Resuspend samples in 100uL of wash buffer (PBS + 10% FBS)

11 Add 1uL of HTO antibodies

12 Incubate for 30 min on ice

13 Add 3.5mL of wash buffer to labeled cells, mix and pellet at 200xg for 10min at 4C

14 Remove supernatant with pipette, resuspend pellet with 3.5mL of wash buffer, and transfer to new tube

15 Pellet at 200xg for 10min at 4C

16 Remove supernatant with pipette and resuspend pellet with 3.5mL of wash buffer

17 Pellet at 200xg for 10min at 4C

18 Assuming 50% cell loss, resuspend pellet in wash buffer to obtain ~1,000 cells/uL

18.1 Count cells and adjust concentration, if needed

10x loading

19 Mix hashtag samples at desired ratio and load 52,500 cells per well