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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

- EasySepTM Dead Cell Removal (Annexin V) Kit (STEMCell Technologies, Cat. # 17899)
- TotalSeqTM-C0262 anti-human Hashtag 12 Antibody (Biolegend, Cat. # 394683)
- TotalSeqTM-C0263 anti-human Hashtag 13 Antibody (Biolegend, Cat. # 394683)
- TotalSeqTM-C0264 anti-human Hashtag 14 Antibody (Biolegend, Cat. # 394683)
- TotalSeqTM-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 Vortex 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