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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **PBMC PROCESSING** | | | | | | | | | | | | | | | | | | | | | **SOP Employed:** CBIG-02-001 | | | | | |
| Processed by: | | | | |  | | | | | | | | | | | | | ID Number: | | | | | | | | |
| Time start processing: | | | | | **:** | | | | | | | | | | | | | Date: **/ /** | | | | | | | | |
| **VACCUTAINERS/TUBES** | | | | | | | | | **QUANTITY** | | | | | | | | | | | | | | | | | |
| **RECEIVED** | | | | **TYPE** | | | | | **RECEIVED** | | | | | | | | | | | | | | | | | |
|  | | | |  | | | | | **mL** | | | | | | | | | | | | | | | | | |
| **Issues** | |  | Red Pellet (RP) | | | |  | | | Clotted | | | |  | Expired tubes Date of expiry: **/** | | | | | | | | | | | |
| **STEPS (if 4 or 5 mL of blood, do the first 3 steps in 15 mL tube and adjust to corrected volume):** | | | | | | | | | | | | | | | | | | | | | | | | | | |
|  | 1. Distribute between 10-15 mL of blood in 50 mL flip cap tube | | | | | | | | | | | | | | | | | | | | | | | | | |
|  | 2. Rinse blood collection tube with PBS, distribute into the flip cap tube and adjust volume to 30 to 35 mL | | | | | | | | | | | | | | | | | | | | | | | | | |
|  | 3. Add between 10-15 mL of Ficoll | | | | | | | | | | | | | | | | | | | | | | | | | |
|  | 4. Centrifuge for 30 minutes at 700Xg, with NO break | | | | | | | | | | | | | | | | | | | | | Start: **:** | | | End: **:** | |
|  | 5. Collect PBMC and put in new tubes and add up to 50 mL of PBS | | | | | | | | | | | | | | | | | | | | | | | | | |
|  | 6. Centrifuge for 15 minutes at 480Xg brake on | | | | | | | | | | | | | | | | | | | | | Start: **:** | | | End: **:** | |
|  | 7. Discard supernatant, loosen pellet, add 10 mL of PBS | | | | | | | | | | | | | | | | | | | | | | | | | |
|  | 8. Count cells | | | | | | | | | | | | | | | | | | | | | | | | | |
|  | 9. Take cell if needed for trizol and complete suspension to 50 mL with PBS | | | | | | | | | | | | | | | | | | | | | | | | | |
|  | 10. Centrifuge for 12 minutes at 310Xg, brake on | | | | | | | | | | | | | | | | | | | | | Start: **:** | | | End: **:** | |
|  | 11. Discard supernatant and loosen cell pellet | | | | | | | | | | | | | | | | | | | | | | | | | |
|  | 12. Add solution A (HI HuAB serum) | | | | | | | | | | | | | | | | | | | | | | | | | |
|  | 13. Add solution B (HI HuAB serum + DMSO) | | | | | | | | | | | | | | | | | | | | | | | | | |
|  | Prepare aliquots | | | | | Date: **/ /** | | | | | | | | | | | | | | | | | | | | |
|  | Store at -80°C | | | | | Date: **/ /** | | | | | | | | | | | | | | | | | | | | |
|  | Store in liquid nitrogen | | | | | Date: **/ /** | | | | | | | | | | | | | | | | | | | | |
| **PBMC COUNTING** | | | | | | | | | | | | | | | | | | | | | **SOP Employed: CBIG-03-001** | | | | | |
| Dilution factor :**2** | | | | | | | | | | | | | Cell suspension volume:**10.00mL** | | | | | | | | | | | | | |
| Countess FL II  (already take into account the dilution factor) | | | | | | | | | | | | | | | | | | | **Count #1** | | | | **x106 cells/ mL** | | | |
|  | | | | | | | | | | | | | | | | | | | **Count #2** | | | | **x 106 cells/mL** | | | |
| SUM OF COUNTS:  Count #1 + Count #2 | | | | | | | | | | | |  | | | | | CELL COUNTING MEAN:  Sum of Count #1 and Count #2 / 2 | | | | | | | | |  |
| TOTAL CELLS ISOLATED : Cell Counting Mean x Cell Suspension Volume (mL) | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Coefficient of Variance (% C.V.)  (need to be under 5%) | | | | | | | |  | | | Live: | | | | | Live: | | | | **Total PBMC** | | | | | | **x106** |
|  | | | | | | | |  | | | Dead: | | | | | Dead: | | | |  | | | | | |  |
| If trizol done take: | | | | | | | | | | | | | | | | | | | | | | | | **x 106 cells** | | |
| **Final PBMC count (after trizol)** | | | | | | | | | | | | | | | | | | | | | | | | **x 106 cells** | | |

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **PBMC FREEZING** | | | | | | | |
| FREEZING SOLUTION B PREPATION (mL)  HuAB serum + 20% DMSO | | HuAB: | **mL** | NUMBER OF VIALS | | |  |
| DMSO: | **mL** |
| TOTAL FREEZING SOLUTION VOLUME USED (mL)  50% Solution A (HuAB serum) + 50% Solution B  Total Volume = 1.00mL x Number of Vials | | | **mL** | CELLS PER VIAL  Total Cells Isolated / Number of Vials  \*\*SHOULD NOT EXCEED 21.50 x106 | | | **x106** |
| **QUANTITY - ALIQUOTS** | | | | | | | |
| X vials at  x 106 cells / mL in each vial | | | | | | | |
| **REAGENTS TRACKING** | | | | | | | |
|  | Lot # | Expiry Date | | | Opened | Finished | |
| PBS |  | **/** | | |  |  | |
| Ficoll |  | **/** | | |  |  | |
| HI HuAB serum |  | **/** | | |  |  | |
| DMSO |  | **/ -** | | |  |  | |
| Vacutainers |  | **/** | | | N/App | N/App | |

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| Notes: |