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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** |

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| **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 BTotal Volume = 1.00mL x Number of Vials | **mL** | CELLS PER VIALTotal 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:   |