HASH Antibodies/FACS Staining prior to sorting

(Adapted from Cell Hashing and Single Cell Proteogenomics Protocol Using TotalSeq Antibodies, BioLegend)

https://www.biolegend.com/protocols/totalseq-a-antibodies-protocol-with-10x-single-cell-3-reagent-kit-v2/5008/

1. After harvesting cells, rinse in 2ml Cell Staining Buffer (BioLegend Cat#420201).
2. Measure volume/cell concentration to calculate total live cell number.
3. Centrifuge at 400g for 5 minutes (or whatever speed/time has been successfully used for the cells of interest before) usually at 4°C in 15ml tube.
4. Remove majority of supernatant. Re-spin sample for an additional 10 seconds to transfer all liquid from inside walls of tube to the bottom. Remove all but approximately 5-10ul of supernatant.
5. Resuspend cell pellet with 75ul of Staining Buffer with 1/20 dilution (3.75ul) Human or Mouse TruStain FcX (Fc Receptor Blocking Solution, Human: BioLegend Cat#422301, Mouse: BioLegend Cat#101320)
6. Incubate for 10 mins at 4°C
7. Prior to addition to the cells the diluted Hashing Antibody is centrifuged at 14,000g for 10 mins at 4°C to remove precipitates When transferring the centrifuged Hashing Antibody to a new tube avoiding the bottom 5ul of the tube with any precipitate that may be there.
8. Add FACS staining antibodies to the Hashing antibody tube and mix.
9. Add the approximately 75ul prepared Hashing/FACS Antibody mix (1ug of hashing antibody for up to 1-2 million cells, made in Cell Staining Buffer) to the cells and gently mix.
10. Incubate for 30 mins at 4°c, gently mixing cells at the half-way point.
11. Wash cells 2 times with 2ml Cell Staining Buffer (400g, 5 mins, 4°C).
12. Resuspend cells in appropriate volume of Cell Staining Buffer for sorting.
13. Sort cells into Eppendorf tube containing 300ul of 0.4% BSA/PBS (or media of choice if the cells are particularly sensitive to extensive periods of time during transportation to Core).
14. Provide to Single Cell Genomics Core for QC, counting, and processing.