HASH Antibody Staining (Post-Sorting) Protocol

(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. Sort gated cells into Eppendorf tubes (DNA LoBind Tubes, Eppendorf cat#022431021) containing 200ul 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 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, and save, all but approximately 5-10ul of supernatant. Removed supernatant (here, and in all subsequent supernatant removal steps) was checked under the microscope for unintentionally removed cells indicative of sub-optimal centrifugation.
5. Resuspend cell pellet with 75ul of Staining Buffer with 1/20 dilution (3.5ul) 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. Add 75ul pre-prepared Total-Seq Hashing antibodies to each cell sample and gently mix (1ug of each, made in Cell Staining Buffer, total volume of 55ul). Prior to addition to the cells the antibody mix is centrifuged at 14,000g for 10 mins at 4°C to remove precipitates, avoiding the bottom 5ul of the tube when removing the antibody mix.
8. Incubate for 30 mins at 4°c, gently mixing cells at the half-way point.
9. Wash cells 3 times with 1ml Cell Staining Buffer (400g, 5 mins, 4°C).
10. Resuspend cells in 0.3ml 0.4% BSA/PBS.
11. Provide to Single Cell Genomics Core for QC, counting, and processing. We will take 15ul to perform live cell count and then pool the hashed cell populations prior to centrifugation and resuspension at 1000 cell per ul in 0.4% BSA/PBS for loading onto 10x Chip