|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Homogenization Buffer**  *(Vortex)* | **6 rxns** | **8 rxns** |  | **Diluent**  *(Vortex)* | **6 rxns** | **8 rxns** |
| 1M Sucrose\* | 3.84 ml | 4.61 ml |  | 1M CaCl2 | 90 ul | 108 ul |
| 1M CaCl2 | 60 ul | 72 ul |  | 1M MgAc2 | 54 ul | 64.8 ul |
| 1M MgAc2 | 36 ul | 43.2 ul |  | 1M Tris pH 7.8 | 180 ul | 216 ul |
| 1M Tris pH 7.8 | 120 ul | 144 ul |  | 0.5M EDTA | 3.6 ul | 4.32 ul |
| 0.5M EDTA | 2.4 ul | 2.88 ul |  | H2O | 2.67 ml | 3.2 ml |
| 10% NP40 | 120 ul | 144 ul |  | Beta ME *(Vortex)* | 1.26 ul | 1.51 ul |
| H2O | 7.83 ml | 9.4 ml |  |  |  |  |
| Beta ME *(Vortex)* | 0.84 ul | 1.01 ul |  |  |  |  |
| RNase inhibitor | 120 ul | 144 ul |  |  |  |  |
|  |  |  |  | **Working Solution** *(Vortex)* | **6 rxns** | **8 rxns** |
|  |  |  |  | OptiPrep | 10 ml | 12 ml |
|  |  |  |  | Diluent | 2 ml | 2.4 ml |
|  |  |  |  |  |  |  |
| **30% OptiPrep** | **6 rxns** | **8 rxns** |  | **40% OptiPrep** | **6 rxns** | **8 rxns** |
| Working Solution | 3.6 ml | 4.32 ml |  | Working Solution | 4.8 ml | 5.76 ml |
| Homogenization Buffer | 2.4 ml | 2.88 ml |  | Homogenization Buffer | 1.2 ml | 1.44 ml |

**ALL BUFFERS AND TISSUE HOMOGENIZERS SHOULD BE ICE-COLD BEFORE USE!**

\*17.1 g Sucrose in 50 ml H2O; filter before use.

1. Add 750 ul 30% solution to a 2 ml dolphin tube; add 300 ul 40% solution to the bottom of the tube.
2. Dounce 50 mg of tissue (or less) in ~700 ul Homogenization Buffer using first the Loose and then the Tight pestle. Recover homogenate and pass through a 40um strainer; add ~450 ul Working Solution. Pipette 10 times to mix.
3. Carefully layer 25% sample dilution on the top of the OptiPrep gradient.
4. Spin at 10,000 g at 4˚C for 5 minutes using fixed angle rotor.
5. Remove upper layer (~700 ul). Recover the nuclear pellet formed on the wall of the tube slightly above the 30%-40% interface; transfer on a new tube.
6. Wash the nuclear pellet with 1 ml 0.04% BSA in PBS.
7. Spin down nuclei at 300 g for 3 minutes at 4˚C. Remove ~950 ul supernatant.
8. When proceeding with the 10x Genomics 3’ snRNA-seq protocol add 1ml 0.04% BSA in PBS; when proceeding with the 10x Genomics Multiome ATAC + GEX protocol add 1ml of the provided DNB as 1x DNB; wash again and spin down nuclei at 300 g for 3 minutes at 4˚C. Remove supernatant leaving the appropriate amount of supernatant.
9. Use C-Chip to count nuclei. Resuspend the nuclei before adding Trypan Blue, usually at a 1:10 dilution ratio. Pipette to mix well and load to the chip chamber.
10. Adjust nuclei concentration as requested per protocol.
11. Ready to proceed with the selected 10x Genomics protocol.