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Preparation of frozen nuclei for single-nucleus RNA sequencing on the 10X Genomics Chromium system

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Start with FAC-sorted nuclei frozen in 1X PBS with 0.8% BSA, 0.5% RNasin Plus RNase inhibitor, and 10% DMSO.

Procedure:

1. Thaw the frozen nuclei in a 37˚C water bath and spin down briefly.
2. Pipette several times to mix.
3. Transfer 8 uL to a Bürker chamber for counting.
4. Load on chip according to the 10X Genomics protocol. If the concentration of the suspension is not high enough to load the 10X, the procedure might be continued as follows:

Nuclei Wash and Resuspension Buffer:
Based on the 10X Genomics protocol for nuclei isolation

	Stock concentration	Final Concentration	2 mL
PBS	1X	1X	1.6 µL
BSA	10% in PBS	2%	400 µL
RNase inhibitor	40 U/µL	0.2 U/µL	10 µL

Procedure:

5. Coat a 1.5 mL low-binding tube with BSA, by dispensing a 30% BSA solution into the tube and then aspirating fully.
6. Add 100-200 uL of Nuclei Wash and Resuspension buffer to the nuclei. Pipette several times to mix.
7. Transfer the nuclei to the low-binding tube that has been coated with BSA.
8. Spin down the nuclei at 500 rcf for 6 min at 4 ˚C.
9. Remove most of the supernatant without disrupting the pellet.
10. Add 30 uL of Nuclei Wash and Resuspension buffer, and resuspend.
11. Transfer 8 uL to a Bürker chamber for counting.
12. Load on chip according to the 10X Genomics protocol.