CAG 10x Genomics Single Cell

10x Genomics Single Cell – live cells

Tissue dissociation: You can use any tissue dissociation protocol, but once you get a single cell suspension, it is recommended to follow the 10x Cell Preparation Guide to perform the cell washing and resuspension in 1X PBS containing 0.04% BSA (400 μg/ml).

Cell suspension volume: Please bring a minimum volume of 60 μL.

Viability: Ideally, input cell suspensions should contain more than 90% viable cells. However, viability of 70% is acceptable. If viability is less than 70%, we would like you to decide whether to proceed or not with your experiment.

Concentration: Ideal concentration range should be between 700 – 1,600 cells/μL.
NOTE: Cell counts from FACS are often overestimated, so please use a different methodology to perform cell counting.

Recommended buffer/media: It is recommended to use 1X PBS (calcium and magnesium free) containing 0.04% weight/volume BSA (400 μg/ml) for washing and resuspension. It is also possible to use the medias listed below with up to 10% FBS or up to 2% BSA to maintain cell health with little to no adverse downstream effects:

1. Dulbecco's Phosphate-Buffered Saline (DPBS)
2. Hank's Balanced Salt Solution (HBSS)

If cell viability cannot be maintained in one of these buffers, it is also possible to wash and resuspend in these medias listed below:

1. Eagle's Minimum Essential Medium (EMEM) + 10% FBS
2. Dulbecco's Modified Eagle Medium (DMEM) + 10% FBS
3. Iscove's Modified Eagle Medium (IMEM) + 10% FBS
4. Roswell Park Memorial Institute (RPMI) + 10% FBS
5. Ham's F12 + 10% FBS
6. 1:1 DMEM/F12 +10% FBS
7. M199

NOTE: Media should not contain excessive amounts of **EDTA** (> 0.1mM) or magnesium (> 3mM) as those components will inhibit the reverse transcription reaction. Media also should not contain antibiotics as this has not been tested in-house by 10x Genomics.

Cell strainer: Filtering cell suspensions with an appropriate cell strainer is a helpful step for removing large clumps and debris. Recommended cells strainers: Flowmi™ Cell Strainer (40 micron) and MACS SmartStrainer (30 micron).

Cell sorting: FACS samples are compatible with the 10x Single Cell workflow. It is possible to sort directly into the media that you will use with the 10x Single Cell Master Mix. Please make sure you communicate with CAG about the sorting conditions. Samples should be washed in case the sorting buffer is EDTA rich.

CAG Sample reception: Deliver your cells to CAG staff during **planned** and **scheduled** hour and date. Hours: Monday - Thursday, **8 am - 3 pm**. Exceptions should be discussed and planned. We will count the cells again before loading the chip for GEM generation, so we kindly ask for your presence during the cell counting to double check the concentration and viability. **PLEASE SCHEDULE WITH WEEKS IN ADVANCE**. CAG offers free of charge mock run/prep check. Mock run is important to make sure your prep is correct and compatible with 10x requirements.

***Important: Keep your samples on ice all the time. ***

For additional questions regarding cell prep, please contact 10x Genomics technical support: support@10xgenomics.com

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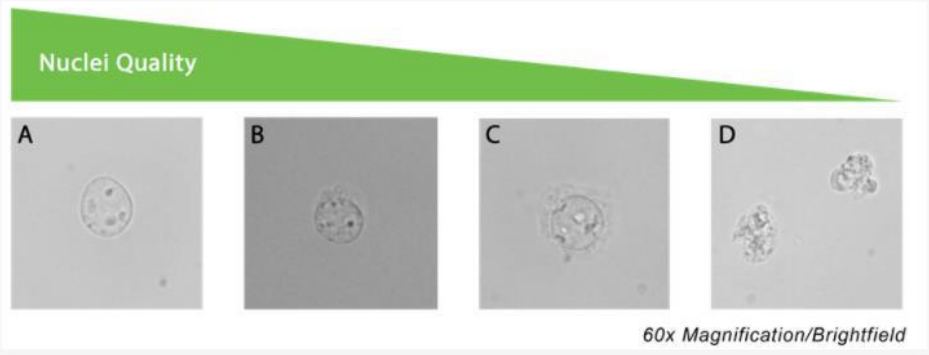
10x Genomics Single Cell – Multiome (3' Gene Expression and ATAC) and scATACseq

Nuclei isolation is a little bit challenging; it will require **major optimizations** on your end, but we can help along the process. We suggest doing the tissue dissociation tests in non-precious samples first, and you can bring them here for us to check the quality. We will stain the nuclei suspension with trypan blue and check it under the microscope. We want to make sure the tissue is properly dissociated, without debris, and most importantly, the nuclei shape needs to be intact.



A: High-quality nuclei have well-resolved edges. Optimal quality for single-cell gene expression libraries.

B: Mostly intact nuclei with minor evidence of blebbing. Quality single-cell gene expression libraries can still be produced.

C: Nuclei with strong evidence of blebbing. ***Proceed at your own risk.***

D: Nuclei are no longer intact. ***Do not proceed!***

CAG offers free of charge consultation and mock runs to check the nuclei prep on the microscope before the real run.

Multiome ATAC + Gene Expression helpful information:
https://www.10xgenomics.com/support/single-cell-multiome-atac-plus-gene-expression

Nuclei Isolation for Single Cell Multiome ATAC + Gene Expression Sequencing:
https://www.10xgenomics.com/support/single-cell-multiome-atac-plus-gene-expression/documentation/steps/sample-prep/nuclei-isolation-for-single-cell-multiome-atac-plus-gene-expression-sequencing

Single Cell ATAC helpful information: https://www.10xgenomics.com/support/single-cell-atac

Nuclei Isolation for Single Cell ATAC Sequencing: https://www.10xgenomics.com/support/single-cell-atac/documentation/steps/sample-prep/nuclei-isolation-for-single-cell-atac-sequencing

CAG Sample reception: Deliver your nuclei to CAG staff during planned and scheduled hour and date. We will count the nuclei again before loading the chip for GEM generation, so we kindly ask for your presence during the nuclei counting to double check the concentration. **PLEASE SCHEDULE WITH WEEKS IN ADVANCE**

Concentration: Ideal concentration range should be between **3,500 – 6,500 nuclei/µL.**

Nuclei suspension volume: Please bring a minimum volume of 30 µL.

Recommended buffer/media: Nuclei should be suspended in a diluted nuclei buffer provided to you by CAG. Please set up a time to pick up the resuspension buffer on the morning of your scheduled experiment.

***Important: Keep your samples on ice all the time.