









## Troubleshooting

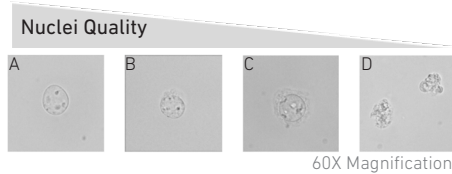
Problem	Possible Solution
High fraction of viable cells post cell lysis	Incrementally increase the lysis time and monitor lysis efficacy microscopically
Difficult to count nuclei/excess debris	Use a fluorescent dye (ethidium-homodimer-1) and fluorescence compatible cell counter or microscope
Excessive debris	Sample may be cleaned by extra washes/ filtering/density centrifugation/FACS (7-AAD stain)
Low nuclei recovery	Use a swing-bucket rotor for centrifugation steps

### Trypan Blue Precipitate in the Countess II Slide



DO NOT use nuclei resuspended in 20X Nuclei Buffer. Repeat nuclei isolation and resuspend in Diluted Nuclei Buffer (1X).

### Nuclei Quality - Representative (Panel A: recommended quality)



- Vortex 0.4% trypan blue stain, centrifuge briefly and aliquot 10  $\mu$ l per tube.
- Pipette mix the nuclei suspension. Immediately add 10  $\mu$ l nuclei suspension to 10  $\mu$ l aliquot of 0.4% trypan blue stain. Gently pipette mix 10x.
- Transfer 10  $\mu$ l trypan blue stained nuclei to a Countess II Cell Counting Slide chamber.
- Insert the slide into the Countess II FL Cell Counter, and determine the nuclei concentration and viability. <5% of input cells should be viable. Optimize focusing and light exposure.

## Nuclei Stock Concentration Table

Based on the Targeted Nuclei Recovery, prepare the nuclei suspension in Diluted Nuclei Buffer to achieve the corresponding Nuclei Stock concentrations.

Targeted Nuclei Recovery	Nuclei Stock Concentration (nuclei/ $\mu$ l)
500	160-400
1,000	320-810
2,000	650-1,610
3,000	970-2,420
4,000	1,290-3,230
5,000	1,610-4,030
6,000	1,940-4,840
7,000	2,260-5,650
8,000	2,580-6,450
9,000	2,900-7,260
10,000	3,230-8,060

DRAFT

## Appendix

### Nuclei Counting and Viability

Countess II FL Automated Cell Counter is recommended for determining nuclei concentrations. The optimal range of cell concentration for Cell Counter is 1,000-10,000 cells/ $\mu$ l. Refer to manufacturer's instructions for details on operations.

### References

- Chromium Next GEM Single Cell Multiome ATAC + Gene Expression User Guide (CG000338)

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