APPLICATION NOTE

Rapid cell count and intactness assessment of pre- and post-Omni Bead Ruptor Elite bead mill homogenized neuroprogenitor cells using the Cellometer K2 fluorescent cell counter.

Summary

Proper cell sample preparation is critical at the front end of any cell-based assays. Specifically, an appropriate cellular lysis procedure is necessary for downstream analyses of intracellular analytes such as DNA/RNA, intracellular proteins, and small molecules (1). However, traditional cellular lysis methods can be time-consuming and heavily reliant on reagents like enzymes or detergents to facilitate lysis. The Omni Bead Ruptor Elite bead mill homogenizer provides a quick and efficient solution for cell lysis that is not dependent on chemical reagents. Cell suspensions can be homogenized in phosphate buffered saline (PBS) on the bead mill in less than 30 seconds, releasing all intracellular analytes of interest into solution and producing homogenate for downstream analysis.

Scientists require accurate cell count and viability results to prepare cells for downstream assays. Cell viability can be measured by assessing the cell membrane integrity with different commercially available viability dyes such as trypan blue (TB), propidium iodide (PI), or a combination of acridine orange (AO) and PI. Nucleic acid staining dyes like AO/PI have been widely used and proven to be a reliable dual-stain for cell counting and viability measurement. AO is an acidic and hydrophobic molecule that readily passes through cellular membranes, binds to double-stranded nucleic acid, and emits a wavelength of 525 nm (green) when excited with a blue light. Contrastingly, PI is impermeable to viable cells with intact membranes and only passes through compromised cellular membranes, where it binds to the nucleic acid and emits a wavelength of 593 nm (red/orange) when excited with a green light (2,3).

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Omni Bead Ruptor™ Elite bead mill homogenizer

Therefore, nucleated cells with intact membranes stained with AO will fluoresce green. In contrast, membrane-compromised cells will only fluoresce red due to Förster resonance energy transfer (FRET) that facilities the absorption of AO fluorescent signals by PI with minimum spill over.

In the past decade, the Cellometer® K2 fluorescent cell counter has been employed for rapid cell count and viability measurement and easily distinguishing nucleated cells from cellular debris and micelles using AO/PI (4,5). This is highly important to reinforce the reliability of upstream cell counting results and membrane intactness assessment, as well as to provide scientists with live cell counts to ensure an appropriate amount of cells is utilized depending on the requirements of downstream assays.

In this work, we demonstrate the use of the Cellometer K2 to determine cell count and viability via AO/PI staining pre-homogenization (intact cells) and post-homogenization (lysed cells) of neuroprogenitor cells produced by the Omni Bead Ruptor Elite bead mill homogenizer. Furthermore, the acquired brightfield and fluorescent images can be used to verify the homogenization and intactness of the cells directly. The cell counter can quickly measure nucleated viable and nonviable cell count results and disregard the non-countable events (debris) during sample preparation that are crucial for the DNA/RNA, intracellular proteins, and small molecule-based downstream assays.

Materials and methods

Equipment

- Omni Bead Ruptor Elite bead mill homogenizer (Cat # 19-042E)
- Omni Bead Ruptor Elite 2 mL Tube Carriage (Cat # 19-373)
- 2 mL Soft Tissue Homogenizing Mix 1.4 mm Ceramic Beads (Cat # 19-627)
- Cellometer K2 fluorescent cell counter (Cat # CMT-K2-S150)
- SD100 Counting Chambers (Cat # CHT4-SD100-002)
- ViaStain™ AO/PI Staining Solution (Cat # CS2-0106-5 mL)

Procedure

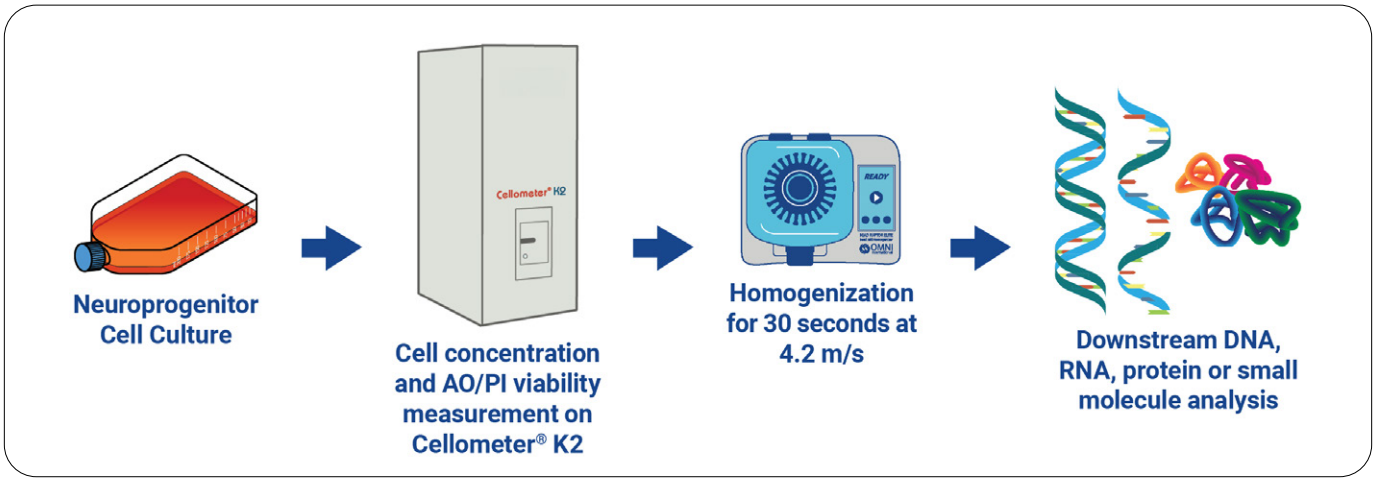

Figure 1: Cell counting and homogenization workflow

Neuroprogenitor cell culture

Neuroprogenitor (NP) cells were obtained from consenting patients per the CIRBI IRB protocol No. Pro00036306. NP Cells were cultured on 75 cm^2 tissue-culture flasks (Corning, Cat # 430641U) coated with 0.1 % vitronectin. Cells were supplemented with Stemflex Medium prepared with 5 % supplement, 2 % L-glutamine (Gemini Bio, Cat # 400-106-100), and 1 % penicillin/streptomycin solution (Gemini Bio, Cat # 400-109). The cells were grown to ~80 % confluency and detached from the flask with Accutase (Gemini Bio, Cat # 400-158). The detached cell solution was centrifuged, where the resulting supernatant was poured off, and the pellet was resuspended in 5 mL PBS, pH 7.2 (Gibco, Cat No. 20012027).

Cellometer K2 Fluorescent Cell Counter

Cellometer K2 (Cat #, CMT-K2-S150) utilizes one bright-field (BR) and dual-fluorescent (FL1 and FL2) imaging to measure the concentration and viability of target cells (Figure 2). The fluorescent imaging used two channels with EX/EM, 470/535 nm and 540/660 nm, for detecting AO/PI fluorescence. The cell sample (20 µL) was pipetted into SD100 counting chambers (Cat # CHT4-SD100-002) with protective films removed from both sides. The counting chamber was inserted into the instrument, where four sets of images (BR, FL1, FL2) were acquired and analyzed by the Matrix software. The BR channel was used for trypan blue-based counting, and FL1/FL2 channels were used for AO/PI-based counting to determine viable and nonviable cell counts.

Cell count and viability measurement of homogenized NP cells

The 5 mL of NP cell suspension was initially counted on the Cellometer K2 by staining the cell suspension 1:1 with a 0.2% trypan blue solution (Gibco, Cat # 15250061) to determine the concentration for homogenization testing. After the initial counting, 500 µL of the NP cell suspension was added to ten separate 2 mL Soft Tissue Homogenizing Mix Tube containing 1.4 mm ceramic beads (Cat # 19-627). Each of the 2 mL tubes was inverted several times to ensure a uniform mixture, and then 20 µL of cell suspension from each bead tube was aliquoted to a separate microcentrifuge tube containing 20 µL of the ViaStain AO/PI Staining Solution (Cat # CS2-0106-5mL). The AO/PI-stained cell sample tubes were placed in a tube rack and stored in the dark at room temperature during cell counting on the Cellometer K2.

The SD100 counting chambers were placed on a fresh Kimwipe after removing the protective films. Next, 20 µL of AO/PI-stained cell sample from each bead tube was loaded into the sample introduction port on each SD100 slide, inserted into the Cellometer K2, and analyzed using the AO/PI assay on the Matrix software. Next, all ten bead tubes containing NP cell suspensions were homogenized on the Omni Bead Ruptor Elite bead mill homogenizer at 4.2 m/s for 30 seconds. Homogenates were then subjected to another round of AO/PI staining and counting on the Cellometer K2 as described previously. The cell count, viability results, and images were assessed to demonstrate the capability of the Cellometer K2.

Results

The Cellometer K2 successfully determined the cell concentration and viability of cultured NP cells pre- and post-homogenization on the Omni Bead Ruptor Elite bead mill homogenizer. Bright and fluorescent images (Figure 3-6) were acquired and analyzed to report the viable and nonviable cell counts/concentrations, which were used to directly calculate cell viability in the software. The images clearly showed that pre-homogenized cell samples have individual and clusters of cells. In contrast, the post-homogenized cell samples did not exhibit any cell-like particles in the images. In addition, it is important to note that the PI fluorescent spots in the fluorescent images without a corresponding intact cell in the brightfield suggested that they were most likely free nuclei released after homogenization.

The average viability results calculated for the four tested NP cell samples was approximately 76 % before homogenization. However, after homogenizing the NP cell samples on the Omni Bead Ruptor Elite bead mill homogenizer and counting on the Cellometer K2, the live cell concentration decreased to 0.00 x 10^6 cells/mL. The viability decreased to 0 % for all four samples, indicating that the homogenizer effectively lysed the majority of the cells present in the sample (Table 1).

Figure 2: Cellometer K2 fluorescent cell counter.

Rapid cell count and intactness assessment of pre- and post-Omni Bead Ruptor Elite bead mill homogenized neuroprogenitor cells using the Cellometer K2 fluorescent cell counter.

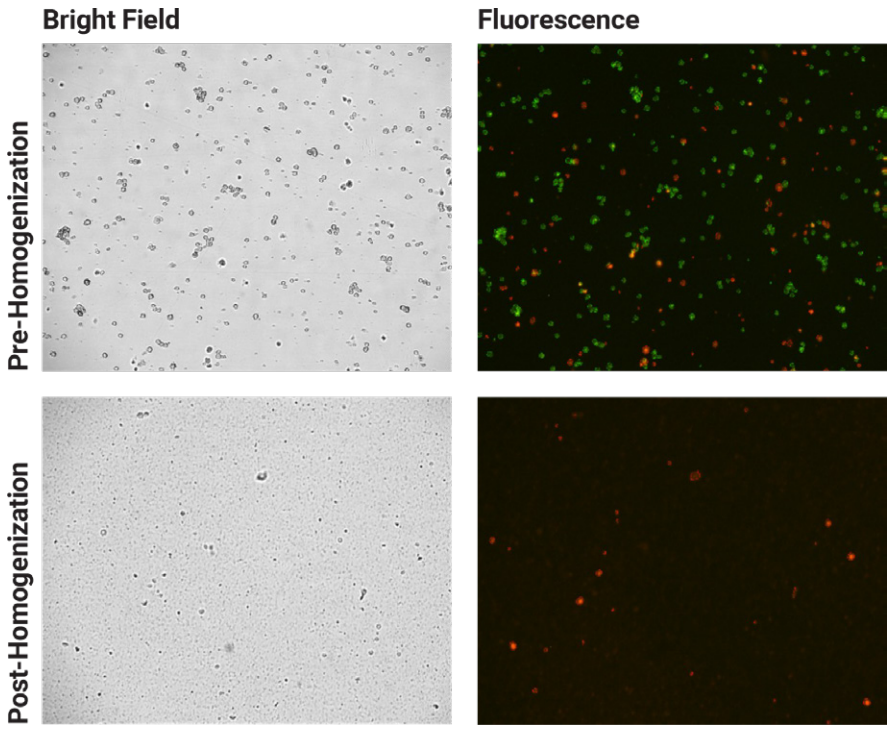

Figure 3: Brightfield and fluorescent images of neuroprogenitor cell sample 1 stained with AO/PI and counted on the Cellometer K2 pre- and post-homogenization on the Omni Bead Ruptor Elite bead mill homogenizer.

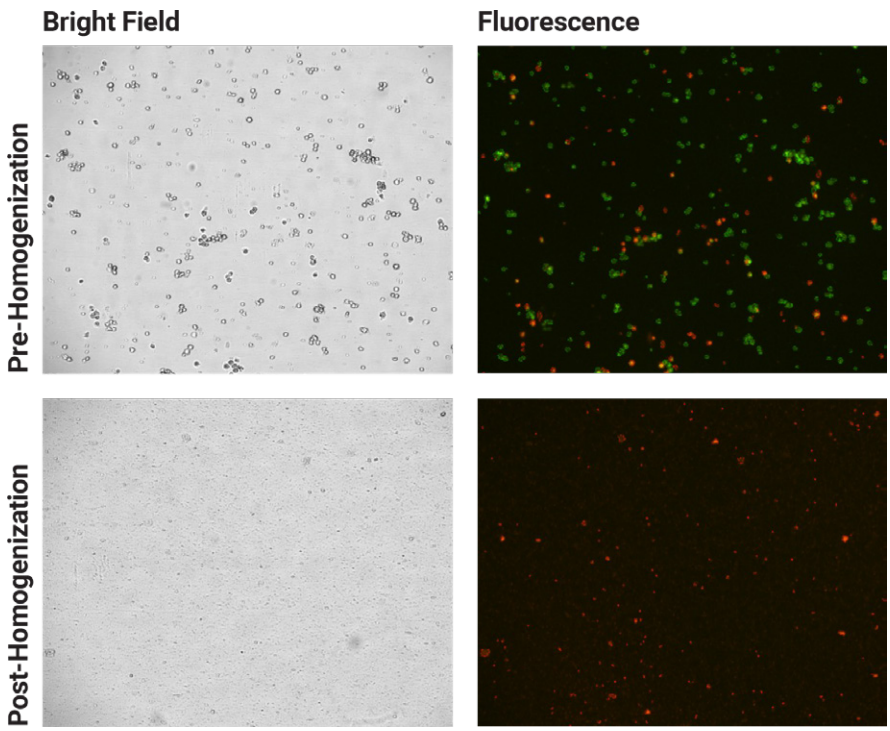

Figure 4: Brightfield and fluorescent images of neuroprogenitor cell sample 2 stained with AO/PI and counted on the Cellometer K2 pre- and post-homogenization on the Omni Bead Ruptor Elite bead mill homogenizer.

Rapid cell count and intactness assessment of pre- and post-Omni Bead Ruptor Elite bead mill homogenized neuroprogenitor cells using the Cellometer K2 fluorescent cell counter.

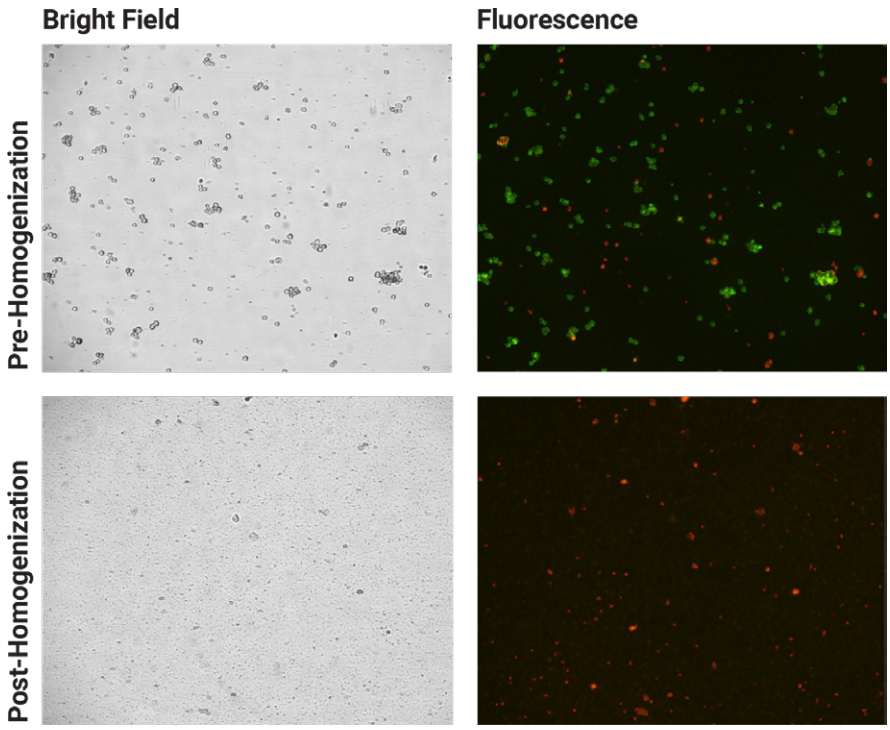

Figure 5: Brightfield and fluorescent images of neuroprogenitor cell sample 3 stained with AO/PI and counted on the Cellometer K2 pre- and post-homogenization on the Omni Bead Ruptor Elite bead mill homogenizer.

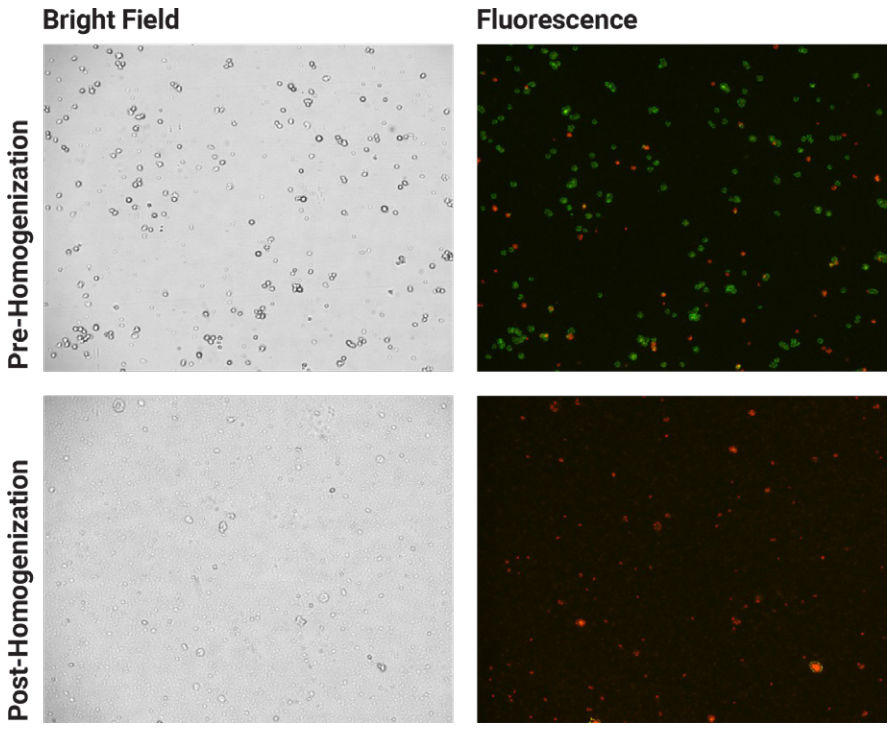

Figure 6: Brightfield and fluorescent images of neuroprogenitor cell sample 4 stained with AO/PI and counted on the Cellometer K2 pre- and post-homogenization on the Omni Bead Ruptor Elite bead mill homogenizer.

Table 1: Cell concentration and viability results of NP cell samples pre- and post-homogenization.

Sample name	Live cell concentration (cells/mL)	Live cell mean diameter (µm)	Viability	Dead cell concentration (cells/mL)	Dead cell mean diameter (µm)	Total cell concentration (cells/mL)	Total cell mean diameter (µm)
NP fresh sample 1	5.30×10^6	12.39	77.0%	1.54×10^6	11.29	6.84×10^6	12.14
NP bead mill sample 1	0.00×10^6	N/A	0.0%	7.01×10^5	9.94	7.01×10^5	9.94
NP fresh sample 2	3.61×10^6	12.69	76.0%	1.15×10^6	11.34	4.76×10^6	12.36
NP bead mill sample 2	0.00×10^6	N/A	0.0%	3.14×10^6	5.24	3.14×10^6	5.24
NP fresh sample 3	3.65×10^6	12.75	76.0%	1.18×10^6	12.2	4.83×10^6	12.62
NP bead mill sample 3	0.00×10^6	N/A	0.0%	2.72×10^6	5.45	2.72×10^6	5.45
NP fresh sample 4	3.40×10^6	13.36	75.0%	1.15×10^6	11.08	4.55×10^6	12.78
NP bead mill sample 4	0.00×10^6	N/A	0.0%	2.19×10^6	5.7	2.19×10^6	5.7

Conclusions

The ability to quickly and efficiently determine cell concentration and viability of cells during the stages of cell preparation is critical for downstream cell-based assays, such as performing plate-based and flow-based cell functional assays, single-cell sequencing, and normalization for metabolic activity and intracellular analyte measurements. In this work, we demonstrated the use of the Cellometer K2 fluorescent cell counter for rapid analysis of cell concentration and viability of pre- and post-homogenized neuroprogenitor cells generated by the Omni Bead Ruptor Elite bead mill homogenizer.

The Cellometer K2 could quickly determine the live and dead cell count by employing the AO/PI dual-fluorescent stain, which only fluorescently labeled nucleated cells or free nuclei. This enabled the cell counter to easily distinguish and count the actual cells instead of cellular debris or micelles. It is important to note that fluorescent cell counting can reliably identify live and dead cells independent of the amount of cellular debris present.

In a previous study, RNA from neuroprogenitor cells was analyzed via RT-qPCR to determine the presence of cyclooxygenase-1 and 2 genes [1]. This workflow included steps to determine cell concentration before sample homogenization and RNA extraction. Proceeding with an RNA extraction workflow without determining cell concentration beforehand can result in an inadequate amount of cells per extraction or, in some cases, an overconcentrated cell sample. Both cases are undesirable and can compromise nucleic acid extraction efficiency and potentially downstream results. These instances are avoidable by using robust cell counting devices like the Cellometer K2 to facilitate the normalization of cell samples upstream of workflows involving RNA or other intracellular analytes.

The Cellometer K2 fluorescent cell counter, combined with AO/PI, provides a robust method for counting the neuroprogenitor cells. In addition, it demonstrated that downstream assays involving the analysis of intracellular analytes could be performed after utilizing the Omni Bead Ruptor Elite bead mill homogenizer, where the cell counter can be used to ensure the cell samples are properly homogenized, releasing DNA/RNA, intracellular proteins, or small molecules.

References

1. Ryan GL, Morehouse ZP, Nash RJ. Quantification of COX-1 and COX-2 Expression from iPSC Derived Neuroprogenitor Cells: Revvity, Inc.; 2021.
2. Ude A, Afi-Leslie K, Okeke K, Ogbodo E. Trypan Blue Exclusion Assay, Neutral Red, Acridine Orange and Propidium Iodide. In: Anil IS, Mansour MA, editors. Cytotoxicity: IntechOpen; 2022.
3. Chan LL, Wilkinson AR, Paradis BD, Lai N. Rapid Image-based Cytometry for Comparison of Fluorescent Viability Staining Methods. Journal of Fluorescence 2012;22:1301-1311.
4. Chan LLY, Laverty DJ, Smith T, Nejad P, Hei H, Gandhi R, Kuksin D, Qiu J. Accurate measurement of peripheral blood mononuclear cell concentration using image cytometry to eliminate RBC-induced counting error. Journal of Immunological Methods 2013;388:25-32.
5. Nair VD, Vasoya M, Nair V, Smith GR, Pincas H, Ge Y, Douglas CM, Esser KA, Sealfon SC. Optimization of the Omni-ATAC protocol to chromatin accessibility profiling in snap-frozen rat adipose and muscle tissues. MethodsX 2022;9.

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