Application note Vol.7

Under Development

Specification and appearance are subject to change without notice

CELLNETTA MZM1 SERIES

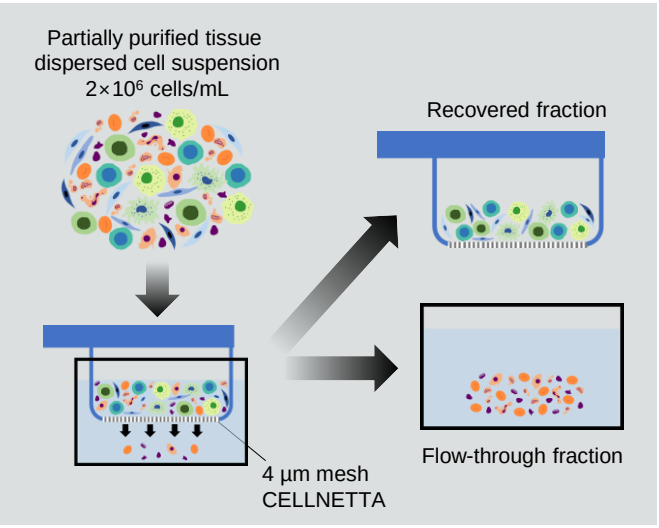
Removal of debris and red blood cells for pre-processing of single-cell analysis

Background

Conventional methods for studying gene expression have been based on analyzing a single sample of a cell population to obtain average data. Single-cell analysis investigates the gene expression of individual cells. Single-cell analysis is applied to research on the diversity of cell populations, cell-cell interactions in diseases, the search for specific marker genes, and the regulatory mechanisms of drug resistance. The most critical factor in obtaining highly accurate data in single-cell analysis is the preparation of a high-quality sample that is free of materials (hereafter referred to as “contaminants”) other than the target cells to be analyzed. The presence of a large amount of cell debris or red blood cells in the sample will impact data reliability during single-cell analysis, leading to lost time and money. The loss of necessary cells during sample preparation is also an issue.
Here, we will introduce a case study of the use of CELLNETTA for the removal of contaminants for pre-processing of single-cell analysis. The study was conducted by Dr. Satoshi Ueha, Associate Professor of the Division of Molecular Regulation of Inflammatory and Immune Diseases at the Research Institute for Biomedical Science at the Tokyo University of Science.

Implementation method

(1) Prepare a partially purified cell suspension of mouse lung tissue dispersed by enzymatic digestion. Then, stain it with various cell surface markers and Calcein-AM, a fluorescent dye for staining live cells.
(2) Apply hydrophilic treatment to the CELLNETTA.*
(3) Place 5 mL of buffer in each of the three wells of a 6-well plate. Place 3 mL of buffer in one well.
(4) Place CELLNETTA in wells containing 5 mL of buffer, and add 1 mL of the cell suspension prepared in (1).
(5) Move CELLNETTA up and down in the buffer 2-3 times, then transfer it to a well containing 5 mL of new buffer. Repeat the process again.
(6) Submerge CELLNETTA in the well containing 3 mL of buffer and allow cells to float.
(7) Pipette the suspension approximately 10 times, then recover 1 mL of the suspension.
(8) Repeat step (7) again to recover a total of 2 mL of cell suspension.
(9) Stain the cell suspension recovered in step (8) with PI (propidium iodide), a fluorescent dye for staining dead cells, then analyze it with a flow cytometer.



* For more information, please refer to the “Hydrophilic Treatment Manual” in the CELLNETTA usage instructions.

Results

Partially-purified cell suspensions of mouse lung tissue dispersed by enzymatic digestion consist of 70.5% contaminants, such as debris and red blood cells (Figure 1(a): Whole). Density gradient centrifugation, which is commonly used to remove such contaminants, reduced the percentage of contaminants to 4.2% after processing (Figure 1(b): Density gradient centrifugation 25/65%). Since there is a difference in size between the contaminants and the target cells, purification using CELLNETTA reduced contaminants to 6.4% (Figure 1(c): 4 µm CELLNETTA). Moreover, a comparison of the cell recovery rate in suspensions that used CELLNETTA and centrifugation with respect to cell suspensions prior to processing indicates that CELLNETTA reduced the loss of each cell type better than density gradient centrifugation (Figure 2).

The processing time of density gradient centrifugation is approximately 40 minutes. However, with CELLNETTA it was possible to reduce this time to less than 10 minutes.

These results indicate that CELLNETTA can be used as a pre-processing tool for single-cell analysis to quickly and accurately remove debris and red blood cells.

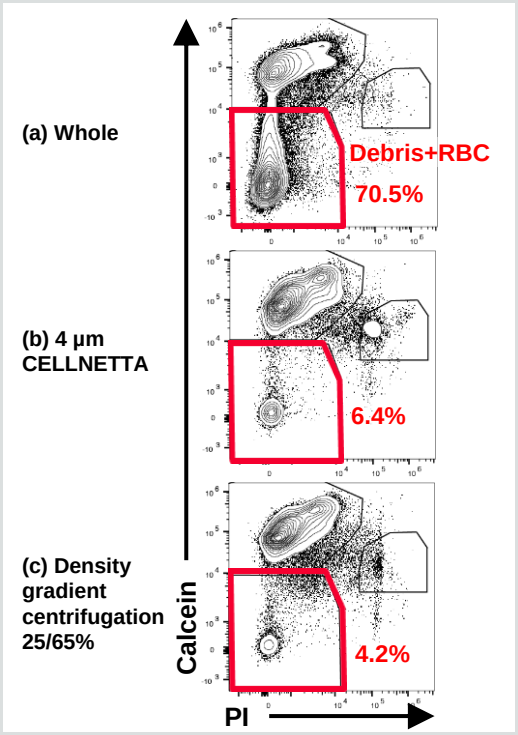


Figure 1 Results of flow cytometry

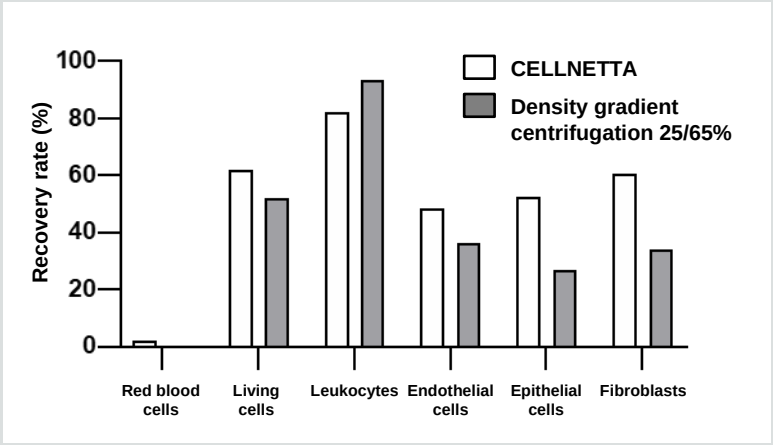


Figure 2 Recovery rate of various cells after processing*

$$\text{*Recovery rate} = \frac{\text{Number of cells in the suspension } \textit{after} \text{ processing}}{\text{Number of cells in the suspension } \textit{prior} \text{ to processing}} \times 100$$

Dr. Satoshi Ueha, Tokyo University of Science; In Preparation.

Product used in this application note

Pore size	Multi well plate model	Product number (P/N)
4 µm	6 well plate	MZMX002B0100
	12 well plate	MZMX006B0100

Notes

- This product is not a medical device.
- This product is a sample for evaluation purpose.
- Please do not ship out your completed product with the sample.
- We shall not be liable for any claims on the sample in case it is shipped out to the market.

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