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Protocol

Protocol of living cell separation using the microfluidic dielectrophoresis integrated chip

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Highlights

Protocol for living cell separation without using biochemical markers

All-in-one microfluid dielectrophoresis integrated chip

A lab-on-chip cell sorting in 30 min with minimum cell damage

Buffer exchange on the chip: no need for sample pretreatment

This protocol demonstrates the separation of living cells with the microfluidic dielectrophoresis chip, using the Jurkat cell as a model. The successful living cell separation lies in familiarity with the detailed tips, which are aided by this stepwise protocol. The knowledge of correct chip installation, sample and buffer filling, flow rate and cell concentration adjustments, and contamination sources increases the efficiency of target viable cell collection. Such instructions, although trivial, are critical for achieving cell separation.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

Protocol

Protocol of living cell separation using the microfluidic dielectrophoresis integrated chip

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SUMMARY

This protocol demonstrates the separation of living cells with the microfluidic dielectrophoresis chip, using the Jurkat cell as a model. The successful living cell separation lies in familiarity with the detailed tips, which are aided by this step-wise protocol. The knowledge of correct chip installation, sample and buffer filling, flow rate and cell concentration adjustments, and contamination sources increases the efficiency of target viable cell collection. Such instructions, although trivial, are critical for achieving cell separation.
For complete details on the use and execution of this protocol, please refer to Oshiro et al. (2022).

BEFORE YOU BEGIN

Separating viable cells from dead cells and non-target cells is essential for research, medicinal, and industrial fields. For instance, separating viable stem cells and their derivatives is crucial for developing stem cell therapies since they can repair and replace damaged cells after injury and disease (Hwang and Lu, 2009; Song et al., 2015). The procedure herein focuses on separating alive Jurkat cells from dead cells.

The present protocol uses a developed chip with a cell-sorting device. This system operates the entire cell sorting process, including sample preparation, buffer exchange, and cell collection with an assigned volume, within 30 min. Therefore, this is a label-free, one-step operation, and a lab-on-chip. Because this system uses microfluidic dielectrophoresis for cell separation, biomarkers and other cell labeling dyes are not needed, minimizing cell damage during the operation.

This chip allows separating the cell size between 10 μm and 35 μm in diameter. The cells below 10 μm diameter are discharged to Outlet port 3, before reaching the dielectrophoresis separation panel of the chip. The width of the flow path of the chip is 50 μm, and therefore, the maximum cell size that can be passed through the chip is around 35 μm. There is no limit to the conductivity of the sample as the sample solvent is replaced with a buffer in this all-in-one operation system.

The cell-sorting parameter must be optimized for target cells before the study. It includes voltage, frequency, flow rate, and cell concentration.

Prepare Jurkat cells in the logarithmic growth phase in RPMI 1640 medium supplemented with 10% fetal bovine serum with 100 U/mL penicillin and 100 mg/mL streptomycin at 37°C in a 5% CO_2 atmosphere. Maintain the culture with approximately 95% viable cells.

Prepare dead Jurkat cells by over-culturing until the survival rate drops to approximately 15%.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Jurkat (human T cell leukemia)	RIKEN BioResource Research Center, Ibaraki, Japan	RCB3052
10% fetal bovine serum	Thermo Fisher Scientific, Waltham, MA	10437
Chemicals, peptides, and recombinant proteins		
RPMI 1640 medium	Thermo Fisher Scientific, Waltham, MA	C11875500BT
Penicillin-Streptomycin solution (x100)	FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan	168-23191
D-PBS(-)	FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan	045-29795
0.4w/v% Trypan Blue Solution	FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan	207-17081
Vacuum desiccator	AFI Corporation, Kyoto, Japan	N/A
Microfluidic dielectrophoresis chip	AFI Corporation, Kyoto, Japan	ECC-001
Buffer for cell sorting	AFI Corporation, Kyoto, Japan	ECB-001
Tube set	AFI Corporation, Kyoto, Japan	ECT-001
Syringe (1 mL)	Terumo, Tokyo, Japan	SS-01T
Software and algorithms		
Cell sorting device designated for the chip (CROSSORTER)	AFI Corporation, Kyoto, Japan	ECS-001
Cell sorter software	AFI Corporation, Kyoto, Japan	N/A
Other		
Automated Cell Counter	Logos Biosystems, South Korea	L10001

STEP-BY-STEP METHOD DETAILS

Setting up instrument

⏱ Timing: 5 min

1. Turn on the computer power switch.
2. Turn on the cell-sorting device power switch.
3. Double-click the cell-sorting device software icon to open the operation screen.

Sample preparation

⏱ Timing: 30 min

In this section, dead and alive Jurkat cells are prepared at 50:50.

4. Equilibrate buffer to room temperature before use to avoid condensation.
5. Equilibrate sample media to room temperature to avoid condensation.
6. Equilibrate alive Jurkat cell culture and dead Jurkat cell culture to room temperature.

Note: It is recommended that samples be at room temperature, consistent with the buffer temperature. A cold buffer and sample can cause condensation and a slow flow rate. It is also necessary to avoid over-equilibration time to maintain the cell's viability.

a. Measure cell density and survival rate with a cell counter.
b. Dilute both cell cultures to approximately 1×10^6 cells/mL with sample media.
c. Mix live and dead cell cultures at approximately 50:50 to prepare 50% viability at 1×10^6 cells/mL (total of alive and dead cells).

Note: The upper limit of cell concentration is recommended at 1×10^6 cells/mL. The cell concentration above the limit can cause inaccuracy in cell counting with the video analysis. Conversely, there is no lower cell concentration limit.

Chip decompression

⏱ Timing: 5 min

In this section, a chip is decompressed immediately before use so that the microchannel of the chip can be adequately filled with fluid.

7. Remove aluminum bad and take out sterile pouch containing a chip.

   Note: The chip is double wrapped, inner with a sterile pouch and outer with an aluminum cover. Remove and discard only the aluminum cover.

8. Place the chip covered with the sterile pouch into a vacuum desiccator.
9. Close the vacuum desiccator lid.
10. Start the vacuum and slowly degas using an arbitrary generator, and leave it for exactly 5 min.

   ⚠ CRITICAL: Over-decompression or insufficient decompression will cause inadequate cell sorting.

11. Turn off the vacuum and slowly release the vacuum state. Wait until the vacuum gauge reaches 0 MPa.
12. Open the vacuum desiccator, and remove the chip covered with sterile pouch.

   ⚠ CRITICAL: After removing the chip from vacuum desiccator, immediately proceed to the next step to set the chip in the cell-sorting device.

Chip installation

⏱ Timing: 2 min

This section introduces chip installation to the cell-sorting device (Figure 1).

13. Open the sterile pouch and take out the decompressed chip.

   ⚠ CRITICAL: After removing chip from sterile pouch, do not touch the inlet and outlet ports of the chip to avoid contamination.

14. Place the chip on the glass stage of cell-sorting device while holding Chip Lock Lever by hand (Figure 2). Ensure that the Inlet 1 and Inlet 2 of the chip are on the left corner.
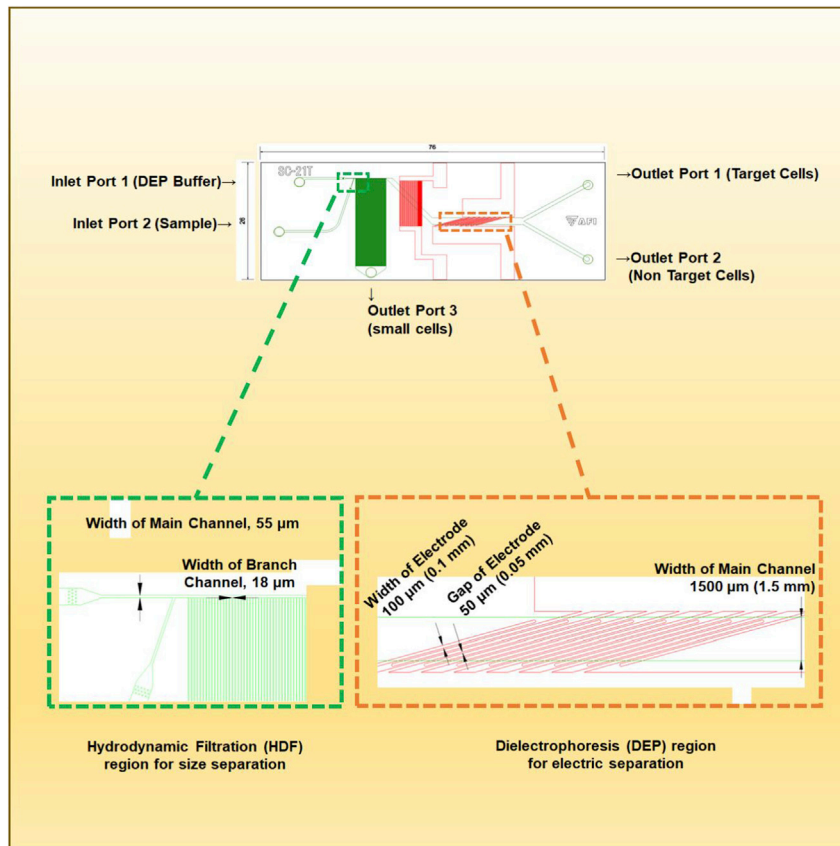
Figure 1. Schematic design of the microfluidic dielectrophoresis chip
The figure is from Oshiro et al. (2022).

15. Push the chip to back unit it latches.

> *Note:* Ensure that Chip Device Lever is up before pushing the chip to back, or the probe unit may be damaged.

16. Lock Chip Device Lever to set the chip position.

Buffer filling

> ⏱ Timing: 5 min

This section introduces buffer filling in the cell-sorting device and chip.

> *Note:* Buffer must be equilibrated at room temperature before use to avoid condensation.

17. Take out the longer tube (buffer tube) from a tube set and connect it to a syringe.

> *Note:* There are two tubes in the kit, one for buffer and the other for sample. Be sure to use the longer one for buffer solution.

18. Manually draw buffer until it reaches 1/3 to 1/2 of the syringe volume.
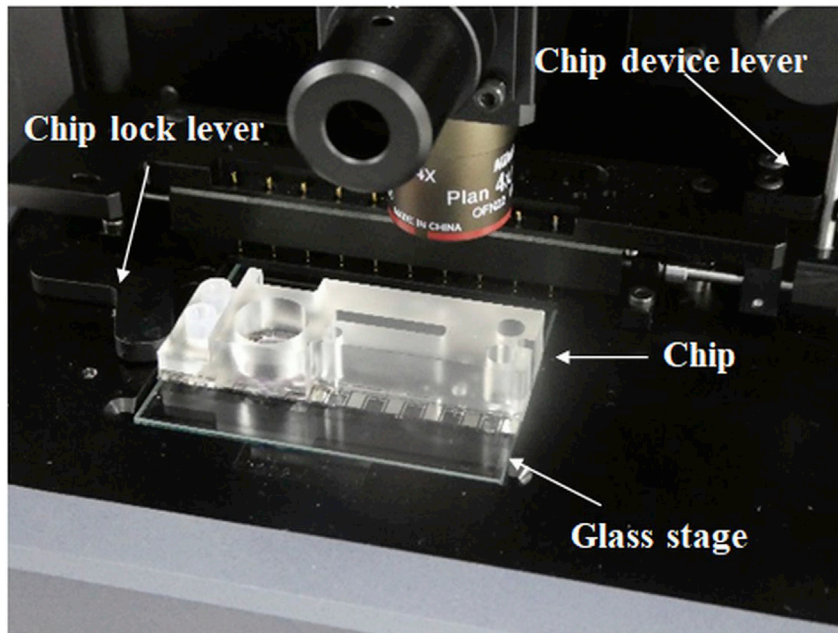
Figure 2. Chip on the glass stage of the cell-sorting device

19. Turn the syringe tip upward and tap the syringe to move air from the tube. Push the piston of the syringe to let all air out.

⚠ CRITICAL: If air bubbles remain in the system, it will affect the constant flow rate.

20. Draw up the buffer to fill the syringe.
21. Install the syringe to Syringe Pump 1 of the cell-sorting device (Figure 3).
22. Insert the other end of the buffer tube into Inlet port 1 attached to the chip (Figure 4).

⚠ CRITICAL: Make sure to insert the buffer tube all the way to Inlet port 1, or the tube can pop off during cell sorting operation.

23. Click Push Plate Fast Descending Button of buffer on the software (6 in Figure 5).

Note: Press once, and the push plate descends and automatically stops when it touches the syringe plunger.

24. Press and hold Push Plate Descending Button (5 in Figure 5) to descend the plunger until the buffer fills the chip and drips from Inlet port 2, Outlet port 1, Outlet port 2, and Outlet port 3.
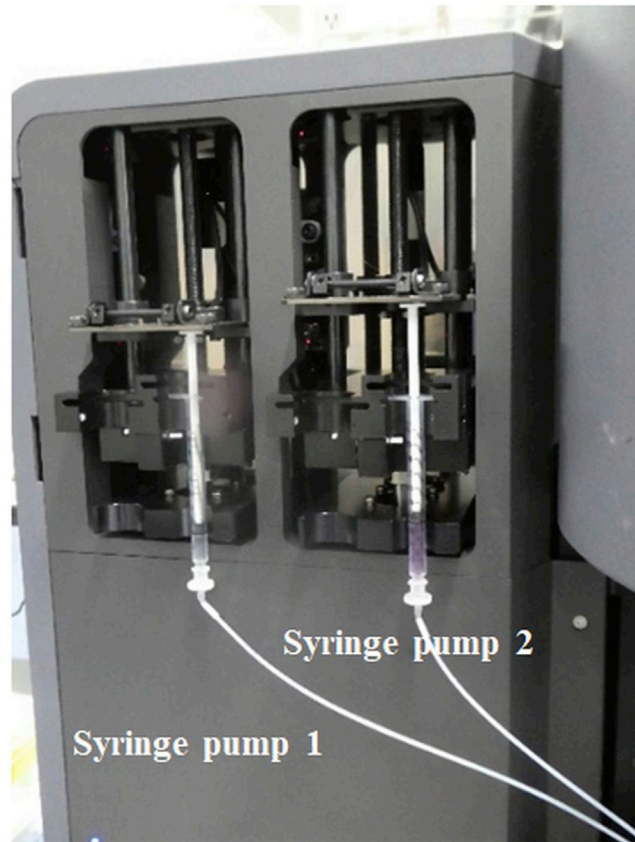
⚠ CRITICAL: Make sure there is no air left in the chip.

25. Keep feeding the buffer in the system for more than 10 min.

⚠ CRITICAL: Successful cell sorting may not be achieved with a shorter conditioning time.

Sample filling

⏱ Timing: 5 min

Figure 3. Syringe Pump 1 and 2 of cell-sorting device
Syringe Pump 1 is for buffer, and syringe Pump 2 is for sample.

This section introduces sample setting.

> *Note:* Sample must be equilibrated at room temperature.

26. Take out the shorter tube (sample tube) from a tube set and connect it to a syringe.

> *Note:* There are two tubes in the kit, one for buffer and the other for sample. Be sure to use the shorter one for sample.

Draw the sample into the syringe in the same procedure as the buffer.

27. Install the syringe to Syringe Pump 2 (Figure 3).
28. Position the sample tube near Outlet port 3 (not shown).
29. Click the plunger Fast descending button of the sample on the software (6 in Figure 5).

> *Note:* Press once, and the push plate descends and stops when it touches the syringe plunger.

30. Press and hold the fluid delivery button on the sample side until a drop comes out from the end of the sample tube (5 in Figure 5).
31. Ensure Inlet port 2 is filled with buffer. Then, insert the sample tube into Inlet port 2.

> ⚠ CRITICAL: Ensure no air bubbles enter into Inlet port 2 (Figure 4).
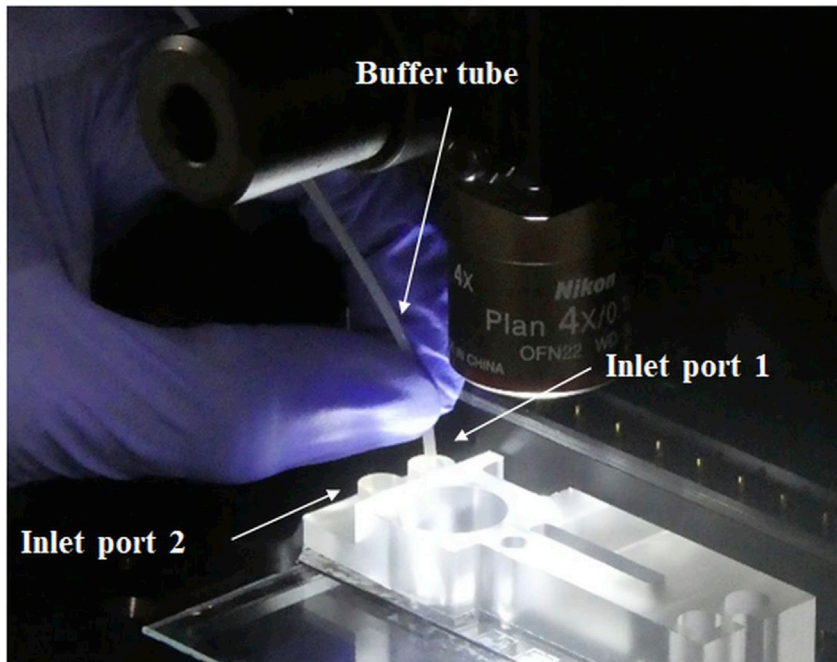
Figure 4. Buffer tube and chip connection
Insert the end of the buffer tube into Inlet port 1 and the sample tube into Inlet port 2.

Microscope setting

⏱ Timing: 5 min

This section introduces the setting of a microscope attached to the cell-sorting device.

32. Adjust screen brightness with Brightness Adjustment Dial of the cell-sorting device located below the syringe pump compartment (not shown).
33. Use Left/Right Adjustment Knob and Front/Rear Adjustment Knob to capture the screen (Figure 6).
34. Focus images with Focus Adjustment Knob (Figure 6).

Cell sorting by manual mode

⏱ Timing: 40 min

This section describes cell sorting operation using manual mode.

Optional: Automatic mode is also available in the software.

35. On the syringe control panel, enter flow rate from the drop-down list (9 in Figure 5).

   Note: Based on the method optimization test, 30 μL/min for 33 min run time is used to separate alive Jurkat cells from dead cells.

36. Set output method for CH1 and CH2 on the waveform control panel (13 in Figure 5).

   Note: Select continuous if target cells are to be collected continuously, which is used for sample enrichment purposes. Otherwise, select ON/OFF.

Figure 5. Syringe control panel and Wave control panel
In the syringe control panel (top), the left side controls buffer and the right side controls sample. Each button function is listed in Table 1.

37. If continuous is deselected, check ☑ and enter liquid feed time (8 in Figure 5).

    Note: In this study, 33 min is entered.

38. Set frequency with ▲ ▼ button (14 in Figure 5).

    Note: In this study, 3,000 kHz is entered. This parameter must be determined by a method optimization experiment for each target cell.

39. Set voltage with ▲ ▼ button (15 in Figure 5).

    Note: In this study, 14 Vpp is entered. This parameter must be determined by a method optimization experiment for each target cell.

40. Press Liquid Transfer Start/Stop button on the syringe control panel for buffer (10 in Figure 5).

    ⚠ CRITICAL: Be sure to start feeding buffer first before sample. To stabilize the flow, a buffer feeding time of 30 s for 1 mL syringe and 5 min for 10 mL syringe is required.

41. Remove buffer accumulated in Outlet port 1, Outlet port 2, and Outlet port 3 using sterile pipettes. Make sure all ports are empty.

Table 1. Cell-sorting device software function buttons

Syringe control panel			
1	Left syringe pump control (buffer)	2	Right syringe pump control (sample)
3	Push Plate Fast Ascending button	4	Push Plate Ascending button
5	Push Plate Descending button	6	Push Plate Fast Descending button
7	Syringe Size (automatic detection)	8	Timer Setting
9	Flow Rate Display and Setting	10	Liquid Transfer Start/Stop button
Waveform control panel			
11	CH1 Waveform Control Panel	12	CH2 Waveform Control Panel
13	Output Selection	14	Frequency Display Setting button
15	Voltage Display, Setting button	16	Apply Start/Stop button
Analysis panel			
17	Analysis Tab	18	File Selection button
19	Branch point setting button	20	Set Analysis Area button
21	Analysis Start (Stop) button	22	Count Reset button
23	Count CSV Output button		

Syringe Control panel buttons # 1–10 refer to Figure 5.

Waveform Control Panel buttons # 11–16 refer to Figure 5.

Analysis Panel buttons # 17–23 refer to Figure 7.

42. Press Liquid Transfer Start/Stop button on the syringe control panel for sample (10 in Figure 5).

   Note: In this study, 1 mL sample (alive cells: dead cells = 50:50 at total 1×10^6 cells/mL, n=3) is passed through the system.

   Optional: Buffer exchange can be recorded by pressing [Start] on the monitoring screen (not shown).
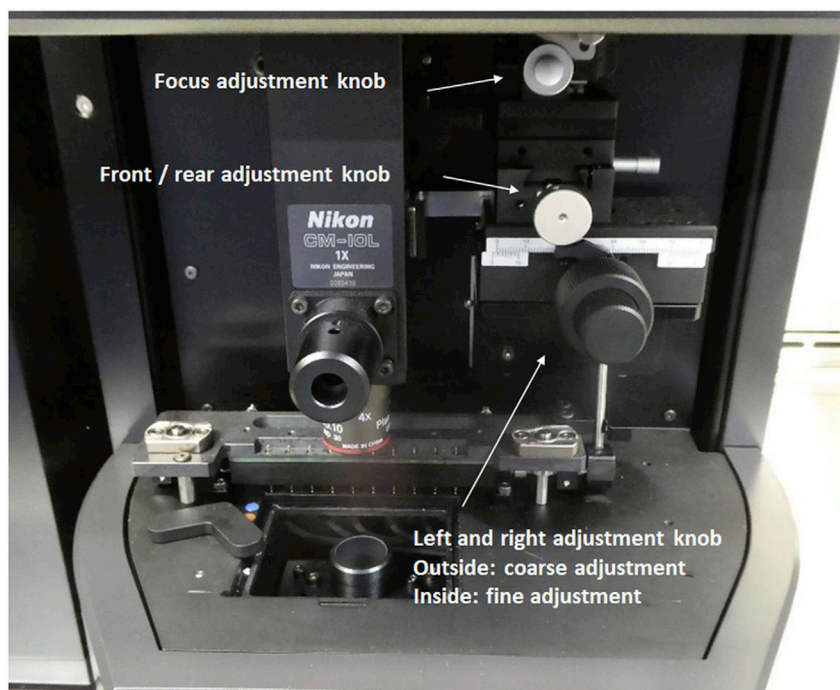
Figure 6. Microscope setting

43. Focus microscope with Focus Adjustment Knob (Figure 6).
44. Press [Start] on the waveform control panel for dielectric separation (16 in Figure 5).

> *Optional:* If necessary, click [Start] on the recording button (not shown). The video is saved in the folder (Windows (C :)> CROSSORTER_WORK> Video) that is automatically created with recording date and time.

45. Using sterile pipettes, transfer separated cells from Outlet port 1 and Outlet port 2 in sterile containers.

> *Note:* Collect solution in Outlet port 1 first.

46. After the target sample volume has passed through the cell-sorting device, press [Stop] on the camera setting panel, waveform control panel, syringe control panel, and solution monitor.
47. Measure cell density and survival rate of sample collected from Outlet port 1 and Outlet port 2 with a cell counter.
48. Measure collected cells in Outlet port 1 and Outlet port 2 using the analysis function of the software (see below section, quantitative analysis).

Quantitative analysis

> ⏱ Timing: 5 min

This section introduces how to analyze cell separation efficiency using a video recording function. This function provides the number of cells captured by Outlet 1 and Outlet 2 in 10 s increments.

49. Press Analysis Tab to display the analysis screen (17 in Figure 7).
50. Click File Selection button to select a video file to be analyzed (18 in Figure 7).
51. Set a branch point (19 in Figure 7).
    a. Set the horizontal line aligned with the middle of flow path branch.
    b. Set the vertical line to the measurement position.

> *Note:* Start with 500 px and move as needed.

52. Set analysis area (20 in Figure 7).

> *Optional:* Analysis can be performed without setting analysis area. Unclick Analysis Area check box if not needed.

53. Click Analysis Start button (21 in Figure 7).
54. Check the degree of cell recognition. Adjust each setting if necessary.
55. Ensure the setting is correct, then press Analysis Stop button (21 in Figure 7).

> *Note:* Once [Analysis start] button is clicked, the same icon changes to [Analysis stop] button.

> *Note:* The count can be reset with Count Reset button (22 in Figure 7).

56. Start cell counting.
    a. Set a play speed of the video and specify the start and stop positions of the video.
    b. Click Analysis Start button again to execute video analysis of the selected section (21 in Figure 7).
57. Press Count CSV Output button on the analysis screen (23 in Figure 7). Save the file (Table S1).

Figure 7. Analysis screen
Each button function is listed in Table 1.

Closing and cleaning

⏱ Timing: 5 min

This section describes cleaning and turning off the system.

58. Remove the syringes from Syringe Pump 1 and Syringe Pump 2 (Figure 3).
59. Remove the tubes from Inlet port 1 and Inlet port 2.

60. Raise Chip Mounting Lever (Figure 2).
61. While pushing Chip Lock Lever by hand, move the chip forward.

    Note: Make sure Chip Mounting Lever is up, or the probe may be damaged.

62. Remove the chip from the glass stage (Figure 2).
63. Click "x" at the top right corner of the operation screen.
64. Turn off the computer.
65. Turn off the power switch on the right side of the cell-sorting device.

Follow-up culture experiment

⏱ Timing: 4 days

This section describes an experiment to confirm that the alive cells are selectively collected through the cell-sorting system.

66. Measure the cell density and survival rate of sample collected from Outlet port 1 and Outlet port 2 with a cell counter.

    Note: The collected cells are in the buffer at this point.

67. Dilute the cells with RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin to approximately 5×10^4 cells/mL. Dispense 1 mL of the culture into 12-wells plates and incubate at 37°C in a 5% CO_2 atmosphere.

    Note: Two controls containing alive cells only are prepared, the Jukart culture with and without passing the cell-sorting system.

68. Every 24 h up to 4 days, record cell counts of the culture prepared from Outlet 1, Outlet 2, and controls.

EXPECTED OUTCOMES

Cell separation efficiency

The anticipated outcome of this protocol is to selectively collect the target cells in Outlet 1. When an AC voltage with a specific frequency is applied to the electrodes, the electrodes capture the target cells flowing from the hydrodynamic filtration (HDF) region (buffer exchange region of the chip) into the dielectrophoresis (DEP) region by dielectrophoretic force, and they are discharged to Outlet Port 1 (Oshiro et al., 2022). The cells that do not respond to a specific frequency are not captured by the electrodes and are discharged to Outlet Port 2. With the example of the above experiment, a culture containing 50% viable Jukart cells was passed through, and the cells collected at Outlet port 1 and Outlet port 2 were measured for cell density and survival rate with a cell counter. The solution collected from Outlet port 1 contained 91.6% alive cells, whereas that from Outlet port 2 contained only 1.8% alive cells (Figure 8). This result indicates that the live Jukart cells react to the set frequency and are adequately sorted.

Cell separation speed and rate

The cells that reached Outlet port 1 and Outlet port 2 were counted in 10 s increment and plotted (Figure 9). The sample before separation contained 49.3% alive Jukart cells with a concentration of 0.96×10^6 cells/mL (total of live and dead cells). One mL of the sample was applied to the cell-sorting system with the above-described parameter. After 1170 s (20 min), a total of 0.61×10^6 cells were collected from Outlet port 1 and Outlet port 2. The average separation rate per 10 s (cell count at Outlet port 1 divided by total cell count collected from Outlet port 1 and Outlet port 2) was 52.5%.
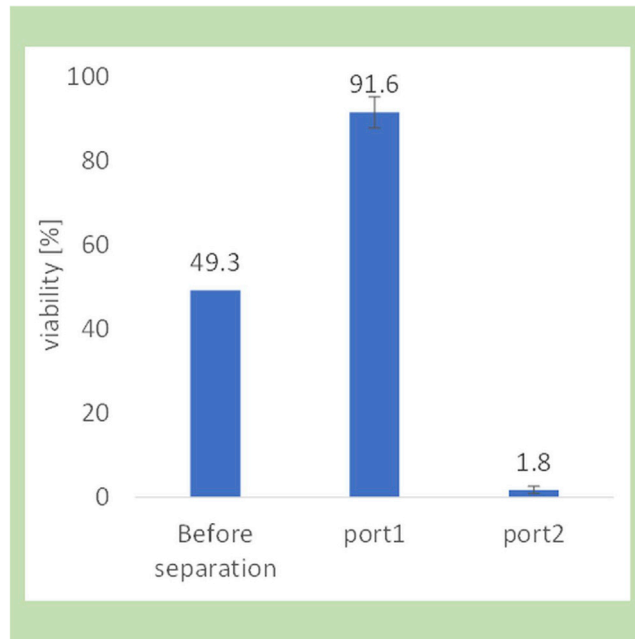
Figure 8. Relative viability of Jukart cell before and after cell sorting
The sample was separated by the cell-sorting system using the parameter of 14 Vpp, 3000 kHz, and 30 μL/min. The solution collected from Outlet port 1 contained 91.6% alive cells, whereas that from Outlet port 2 contained < 2% alive cells.

These results indicate that the 50% viable Jukart cell culture is separated at a constant separation rate, and the entire separation is completed in less than 30 min.

Cell separation confirmation

The follow-up experiment was performed to confirm that the cells collected at Outlet Port 1 (91.6% viable) would be maintained alive after passing through the cell-sorting system. The cells collected at Outlet Port 1 were grown in the culture media for four days, resulting in a growth rate equivalent to the control (Figure 10). On the contrary, the cells collected at Outlet Port 2 (1.8% viable) did not increase the cell count over the four days. The control culture containing nearly 100% viable Jukart cells was passed through the cell-sorting system and the cells collected at Outlet Port 1 were grown in the culture media. The growth rate of this control was equivalent to that of the culture without passing through the system. These results indicate that the cell-sorting system does not damage Jukart cells, and the products after passing through the system maintain the cell's viability.

LIMITATIONS

This protocol separated the alive Jukart cells from dead cells efficiently and adequately with the selected parameters. However, the collected cells at Outlet port 1 contained 8.4% of dead Jukart cells. Whether 91.6% efficiency can be considered acceptable criteria depends on how the product cells are to be used. The dead cells cannot react on the frequency. Therefore, it is likely that these cells died after being collected at Outlet port 1. However, the reason for the 8.4% cell death at Outlet port 1 is uncertain. The possible explanation can be that these cells may have been in the late stationary phase when they entered the system, and the sudden environment change (buffer exchange and electric implement) may have resulted in death after reaching Outlet port 1.

This protocol can be applied to any cell separation practice as long as the target cell possesses different sizes or conductivity from non-target cells. However, the separation parameter described here is specific for Jukart cell separation, and the parameter must be optimized depending on the target cells because each cell has a different size, shape, and conductivity. In addition, sample
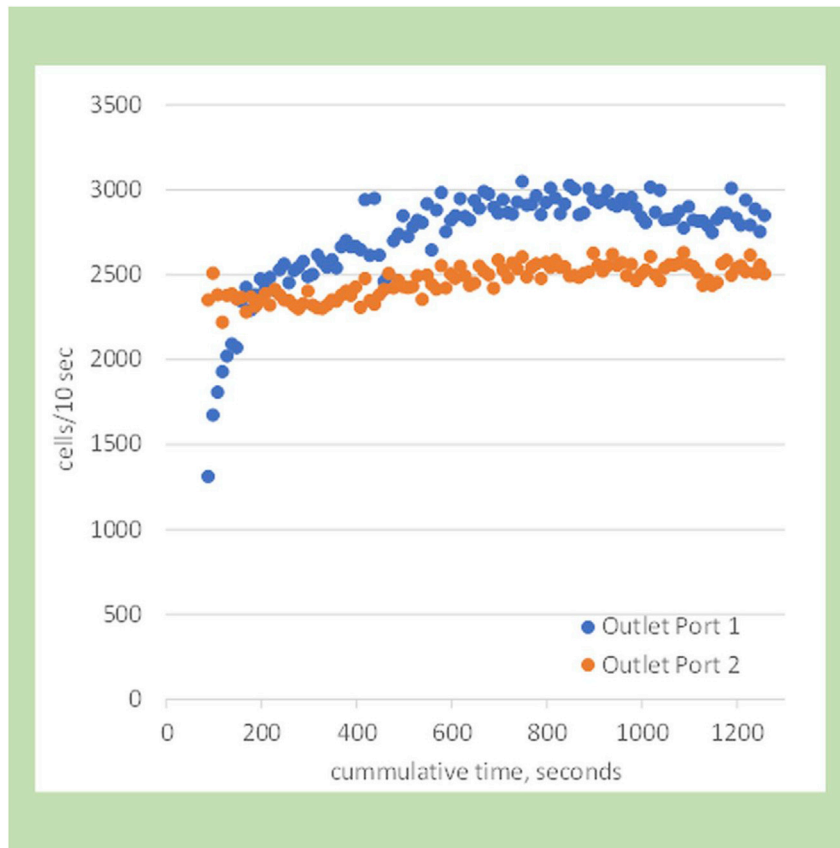
Figure 9. Cell count per 10 s at Outlet port 1 and Outlet port 2
The sample was 49.3% alive Jukart cells with 0.96 × 10^6 cells / mL. One mL of the sample was separated by the cell-sorting system using the parameter of 14 Vpp, 3000 kHz, and 30 μL/min. After 1170 s, a total of 6.1 × 10^5 cells were collected from Outlet port 1 and Outlet port 2.

concentration and flow rate are important parameters to decide, as an overly concentrated sample with a fast flow rate can clog the system. Therefore, a preliminary study is required to select voltage, frequency, sample concentration, and flow rate.

This chip has a cell size limit between 10 μm and 35 μm in diameter. The cells below 10 μm diameter can be applied to the system but are discharged to Outlet port 3, before reaching the dielectrophoresis separation panel of the chip. The width of the flow path of the chip is 50 μm, and thus the upper limit of cell diameter is approximately 35 μm diameter.

This protocol used buffer ECB-001. However, this buffer may not work for other cell types. For example, it was discovered that phytoplankton cells needed osmotic pressure adjustment in the buffer, or the cells were raptured during the buffer exchange stage. Therefore, an excipient that does not possess high conductivity is added to this buffer for phytoplankton cell separation.

This is a disposable chip. A new chip should always be used to avoid contamination and maintain cell separation at a constant flow rate. The price of a chip is approximately a hundred US dollars. Together with the cell sorting device and software, cell sorting could be costly.

TROUBLESHOOTING
Problem 1
The cell sorting rate is inconsistent (steps 35–48).
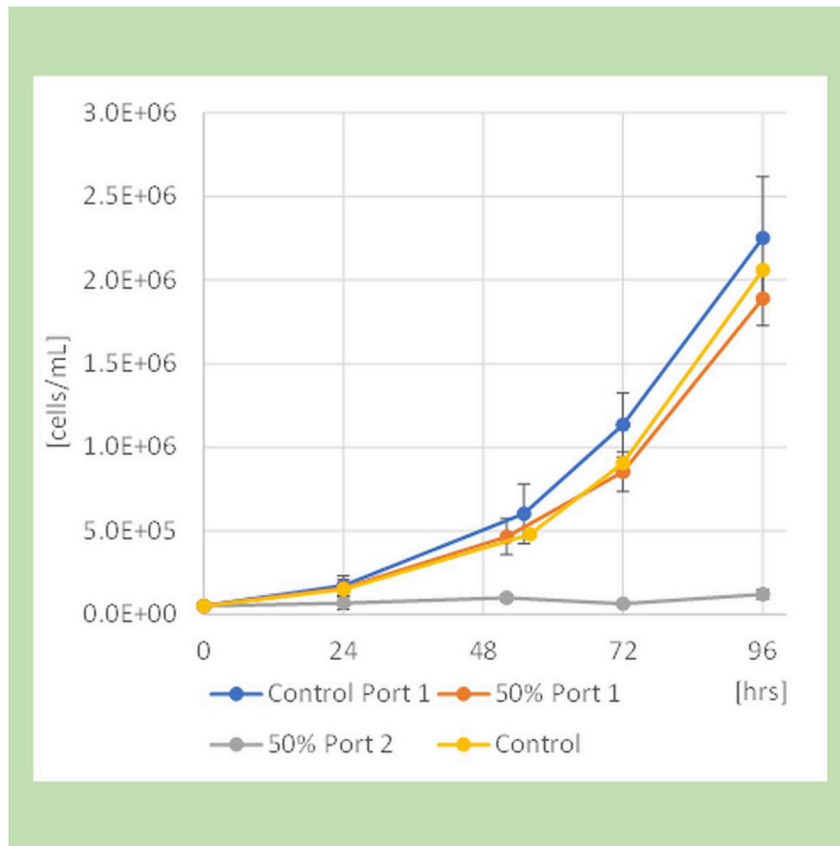
Figure 10. Jukart cell growth curve for four days
The cells captured at Outlet port 1 and Outlet port 2 were grown in the culture media over four days. The control was a Jukart culture with and without passing through the cell sorting system. The control passed through the system was collected from Outlet Port 1 and grown in the culture media.

Potential solution
Over-decompression or insufficient decompression of the chip will cause inadequate cell sorting. After decompressing the chip with a vacuum, the fluid must be filled in the chip immediately. A cold buffer and sample can cause condensation and slow down the flow rate. It is recommended that buffer and samples be at room temperature. However, care must be taken not to over-equilibrate to maintain the sample cell's viability.

Problem 2
The output cells are contaminated (steps 35–48).

Potential solution
Use a new chip every time. After removing the chip from the sterile pouch, do not touch the inlet and outlet ports to avoid contamination. Use sterile pipettes to collect product solutions.

Problem 3
The flow rate is unstable (steps 35–48).

Potential solution
Air bubbles must be removed from the system. Ensure to insert syringe filled with liquid without bubbles, and insert the tubes filled with buffer and sample all the way to Inlet ports.

Apply exact conditioning time to flush the system. Be sure to start feeding buffer first before sample. To stabilize the flow, a buffer feeding time of 30 s and 5 min is required for a 1 mL syringe and a 10 mL syringe, respectively.

Problem 4
Is this a Chip or Cell-sorter problem? (steps 35–48).

Potential solution
When cell separation is not adequately performed, find whether the problem is due to the cell-sorter or the chip. This chip can be operated without the cell sorter. Test the chip manually by applying a sample with a syringe and collecting products from Outlet 1 and Outlet 2 using a peristaltic pump. If the product cells pass through the system properly, the problem probably lies in the cell-sorter setup. Conversely, note that this cell-sorting device works only with the designated chip.

Problem 5
The chip is clogged (steps 35–48).

Potential solution
Cell diameter over 35 μm cannot be applied to this system. The sample cell concentration must not exceed 1×10^6 cells/mL. Dilute the sample with an appropriate media to 1×10^6 cells/mL or less. Ensure that a chip is decompressed, or the microchannel of the chip cannot be adequately filled with fluid, causing cell clogging. Ensure air bubbles are removed from the system.

RESOURCE AVAILABILITY

Lead contact
Further information and other requests should be directed to and will be fulfilled by the lead contact, Fumito Maruyama (fumito@hiroshima-u.ac.jp).

Materials availability
The developed microfluidic dielectrophoresis integrated chip, CROSSORTER, and Buffer ECB-001 are available from AFI Corporation, Kyoto.

Data and code availability
All data produced in this study are included in the published article, and its supplementary information is available from the lead contact upon request. This paper does not report the original code.

SUPPLEMENTAL INFORMATION
Supplemental information can be found online at https://doi.org/10.1016/j.xpro.2022.101527.

AUTHOR CONTRIBUTIONS
All authors reviewed the manuscript and approved the final article. In addition, K.K., K.O., Y.W., and M.T. contributed to the laboratory experiment and data collection. K.K., K.Y., and S.F. contributed to data analysis and manuscript writing. F.M. supervised the project. The work done by K.K., K.Y., S.F., and F.M. was supported by a grant (JPMJSA1705) for a study on Science and Technology Research Partnership for Sustainable Development-Monitoring Algae in Chile (SATREPS-MACH).

DECLARATION OF INTERESTS

We have no financial interest to declare. It should be noted that K.O., Y.W., and M.T. are employees of AFI Corporation.

REFERENCES

Hwang, D.F., and Lu, Y.H. (2000). Influence of environmental and nutritional factors on growth, toxicity, and toxin profile of dinoflagellate *Alexandrium minutum*. Toxicon *38*, 1491–1503. https://doi.org/10.1016/s0041-0101(00)00080-5.

Oshiro, K., Wakizaka, Y., Takano, M., Itoi, T., Ohge, H., Koba, K., Yarimizu, K., Fujiyoshi, S., and Maruyama, F. (2022). Fabrication of a new all-in-one microfluidic dielectrophoresis integrated chip and living cell separation. iScience, 103776.

Song, H., Rosano, J.M., Wang, Y., Garson, C.J., Prabhakarpandian, B., Pant, K., Klarmann, G.J., Perantoni, A., Alvarez, L.M., and Lai, E. (2015). Continuous-flow sorting of stem cells and differentiation products based on dielectrophoresis. Lab Chip *15*, 1320–1328. https://doi.org/10.1039/c4lc01253d.