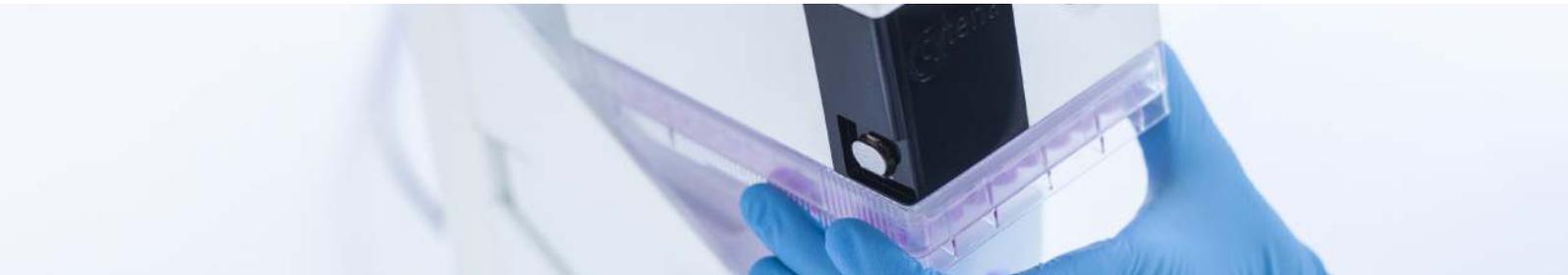


# The C.BIRD: An Innovative Cell Culture for Human T Cells

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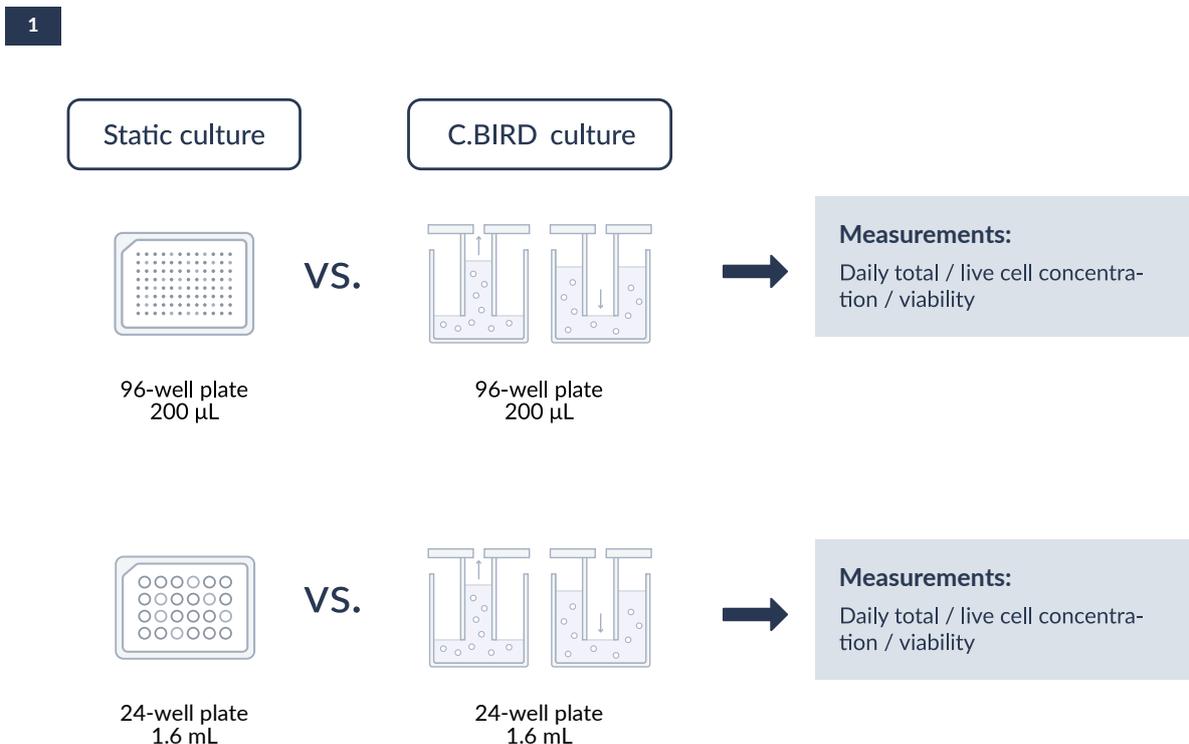
## Abstract

Immunotherapy uses a person's own immune system to fight cancer. As immunotherapy has become a popular cancer treatment, researchers have developed different kinds of immunotherapies. One of them is genetically engineered T cells, such as chimeric antigen receptors (CARs) T-cell therapy (CAR T-cell therapy). In this study, we aimed to improve the cell proliferation rate of human T cells in 96-well and 24-well plates by introducing a novel C.BIRD cell culture method. Our C.BIRD method provides automatic agitation in each well, creating a suspension culture environment and better circulation of nutrients in the medium. In this application note, the Jurkat cell line was used to represent human T cells and cultured using C.BIRD and static methods to compare cell growth. Our results show that the C.BIRD cell culture method improves human T-cell growth without compromising the cell viability when compared to the static culture method. Our C.BIRD technology demonstrates great potential to improve T-cell therapy studies.

# Introduction

Immunotherapy is a crucial advancement in cancer treatment. However, the efficacy of immune checkpoint inhibitors depends on the presence of sufficient tumor-specific lymphocytes. To make up for this shortcoming, cellular immunotherapies have recently produced very promising results. T cells genetically engineered to express CARs have impressive therapeutic effects in some patients with certain subtypes of B-cell leukemia or lymphoma. Nevertheless, various challenges, such as other cancer types, severe toxicities, restricted trafficking of modified T cells to tumors, infiltration into tumors and activation within tumors, hamper the efficacy and prevent the widespread use of CAR T-cell therapies. Speeding up the research and development process of cellular immunotherapies might increase the chance of finding better regimens. The typical CAR T-cell therapy research workflow includes CAR design and lentivirus expression, isolation of donor T cells, followed by efficient activation, transduction of the CAR construct, CAR T-cell expansion as well as functionality and antigen specificity assessments of the final cell product. Accelerating the CAR T-cell expansion step of the workflow may significantly reduce the development time.

We have already published that the C.BIRD microbioreactor for 96-well and 24-well plates showed remarkable improvements in cell growth of Chinese hamster ovary (CHO) cells ([C.BIRD- cell line development](#)). In this study, we proceeded to optimize the T-cell growth in the C.BIRD culture system. We used the Jurkat cell line to represent the human T cells and found that the gentle mixing mode improved the growth rate of the Jurkat cell line compared to the standard static culture, providing an ideal cell culture condition for the human T cells and a more effective workflow for the development of CAR T-cell therapy.

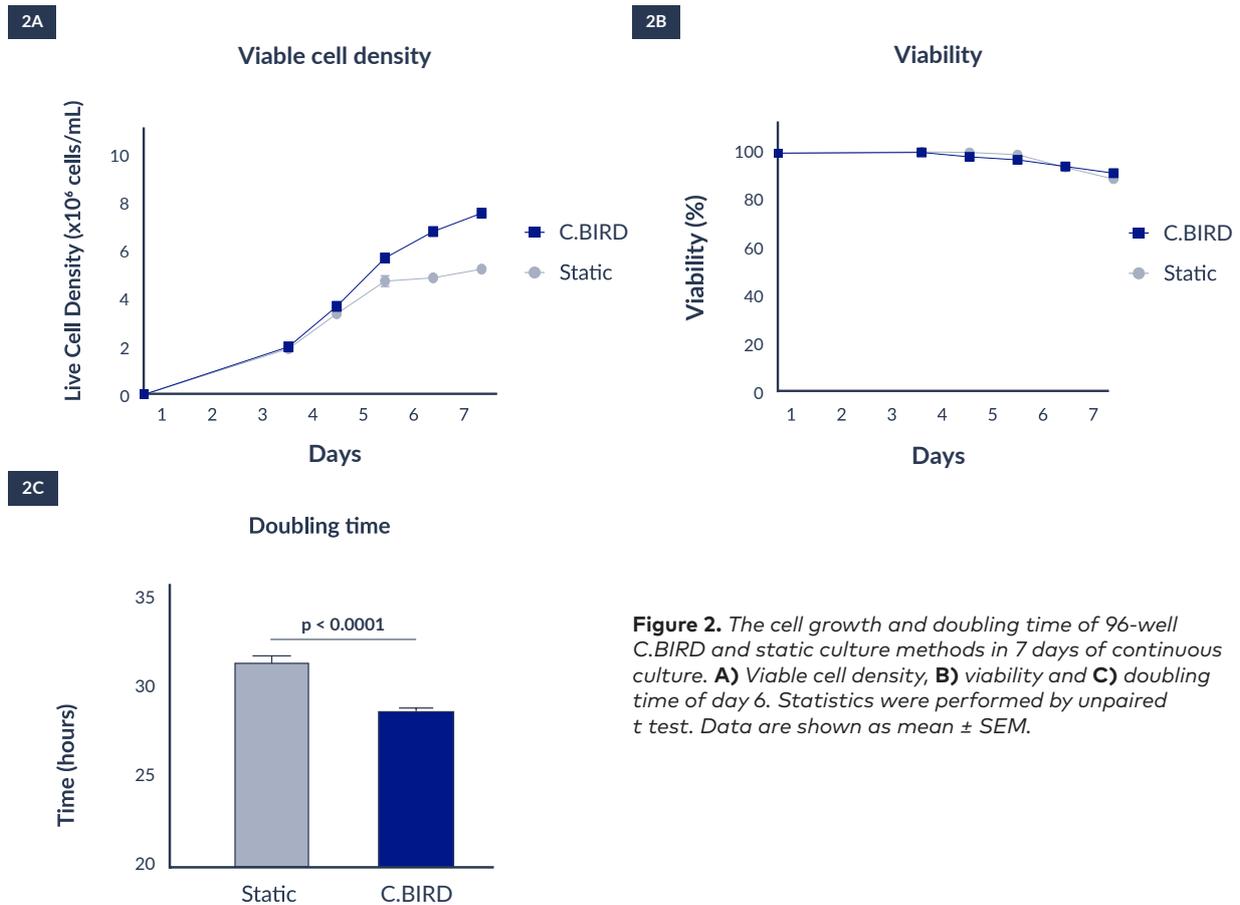


**Figure 1.** Diagram of experiment design.

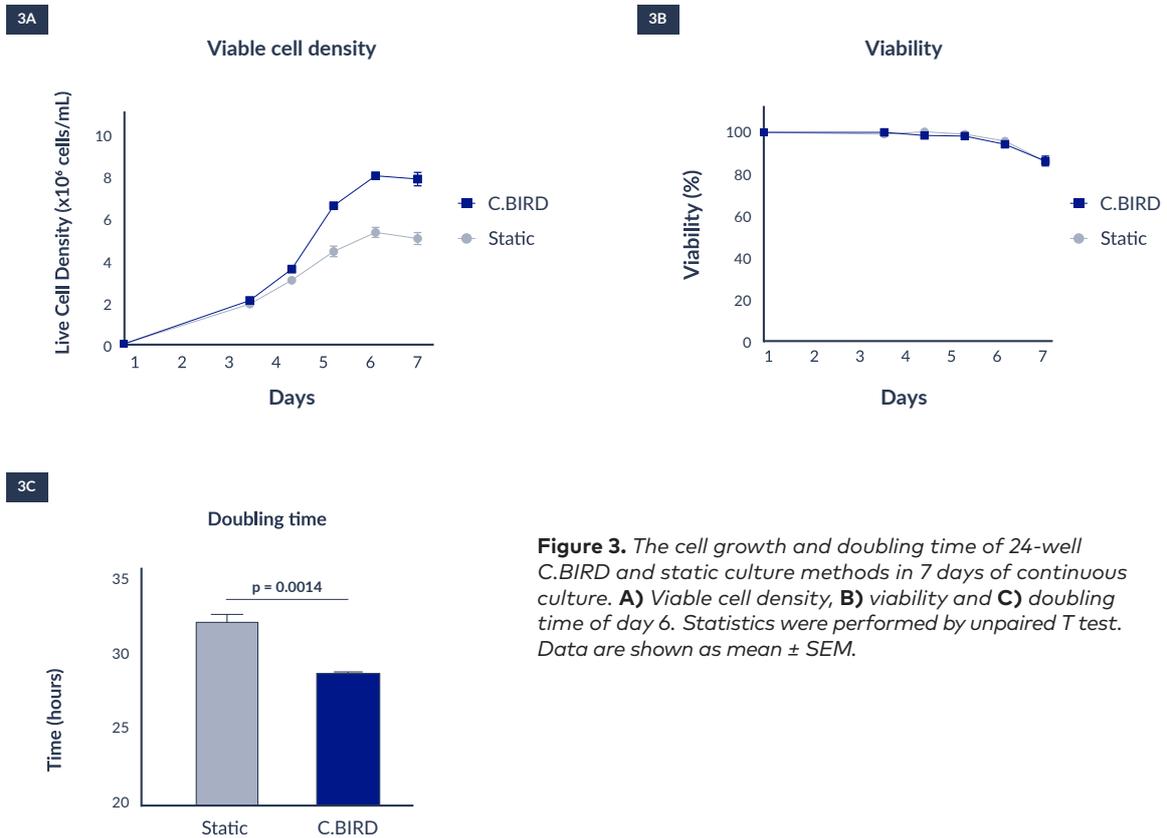
# Materials and methods

The Jurkat cell line, an immortalized line of human T lymphocyte cells, was the model used in this study. The Jurkat cell was cultured in RPMI 1640 medium (#10-040-CV, Corning) supplemented with 10% FBS (#10437028, Gibco), 2 mM L-glutamine (#25-005-Cl, Corning), 10 mM HEPES (#15630080, Gibco), 1 mM sodium pyruvate (#11360070, Gibco), 2,500 mg/L glucose (#G7021, Merck) and 1 x penicillin-streptomycin solution (#30-002-Cl, Corning). The standard 24-well plates (#662102, Greiner) and 96-well plates (#0030 730.011, Eppendorf) were used for this experiment. Comparison studies were performed with cells cultivated in standard static culture and C.BIRD suspension culture in a 37°C, 5% CO<sub>2</sub> incubator environment.

The initial cell concentration was  $2 \times 10^5$  cells/mL in all cell culture methods. The volume of both the static and the C.BIRD groups was 200  $\mu$ L/well medium in a standard 96-well plate, and 1,600  $\mu$ L/well medium in a standard 24-well plate. The mixing rate was 40s/cycle with continuous mixing mode. The total cell density, viable cell density and cell viability were counted with an automated cell counter (TC20, Bio-Rad) from day 3 to day 7 in triplicate. The doubling times were calculated on day 6. Cell growth and doubling times were compared. Data were analyzed by an unpaired *t* test. The significance of *P* values is listed as the following: *P* > 0.05 (ns), *P* ≤ 0.05 (\*), *P* ≤ 0.01 (\*\*), *P* ≤ 0.001 (\*\*\*). Data are shown as mean ± SEM.



**Figure 2.** The cell growth and doubling time of 96-well C.BIRD and static culture methods in 7 days of continuous culture. **A)** Viable cell density, **B)** viability and **C)** doubling time of day 6. Statistics were performed by unpaired *t* test. Data are shown as mean ± SEM.



**Figure 3.** The cell growth and doubling time of 24-well C.BIRD and static culture methods in 7 days of continuous culture. **A)** Viable cell density, **B)** viability and **C)** doubling time of day 6. Statistics were performed by unpaired T test. Data are shown as mean  $\pm$  SEM.

## Results and discussion

The C.BIRD method provides perpendicular mixing in multi-well plates, which introduces better circulation of nutrients and oxygen because of the constant flow. To evaluate if the C.BIRD method with gentle mixing mode can improve human T-cell growth, we conducted an experiment as shown in **Figure 1**.

In the 96-well plate scale, the average viable cell density of cells cultured using C.BIRD's continuous mixing (40s/cycle) reached  $7.53 \times 10^6$  cells/mL on day 7, which was 1.43-fold higher than that of the static group (**Figure 2A**). The cell viability of the 96-well C.BIRD group was 89%, while the static group was 86.3% on average (**Figure 2B**). From the improvement in cell growth in the C.BIRD culture, the doubling time of the C.BIRD culture and static culture on day 6 were 28.32 and 31.18 hours on average, respectively (**Figure 2C**). The doubling time of the C.BIRD group was significantly shorter than that of the static group (*P* value < 0.0001).

In the 24-well plate culture scale, the continuous mixing (40s/cycle) culture condition of the C.BIRD also showed improvement in cell growth. The average of viable cell density was  $6.99 \times 10^6$  cells/mL on day 7, which was 1.53-fold higher than that of the static group (**Figure 3A**). The cell viability of the C.BIRD group was 82.6%, while the static group was 82.3% on average (**Figure 3B**). The doubling time of the C.BIRD group and the static group were 27.92 hours and 31.44 hours, respectively (**Figure 3C**). The result of cell doubling times demonstrated that the growth rate of the Jurkat cell was significantly improved by the 24-well C.BIRD culture (*P* value < 0.01).

These data show the C.BIRD culture method can improve human T-cell growth without compromising cell viability in both 96-well and 24-well systems.

# Conclusion

These results show the C.BIRD culture improved the culture environments in 96-well and 24-well culture plates by increasing the cell growth rate (higher cell density and shorter cell doubling time) of the Jurkat cell, a human T-cell line, without affecting cell viability. The C.BIRD method provided an innovative way to boost human T-cell growth.

Immunotherapies, such as genetically engineered T-cell therapy, have emerged as the most promising areas of cancer research in recent years. Here, our C.BIRD culture system optimizes human T-cell culture with a higher cell proliferation rate, potentially accelerating the research and development process of T-cell-related immunotherapies.

# References

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