

# C.BIRD™ | Enhancing the Cell Growth and Protein Productivity of Adherent HEK293 Cell Line in 96- and 24-well plates

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## Abstract

The human embryonic kidney 293 cell line (HEK293) and its derivatives are among the most important cell lines in the biotherapeutic and academic fields. These cell lines are often used as protein or viral vector producing cells. This study demonstrated the C.BIRD™ microreactor's ability as a novel culture method to improve adherent HEK293 cell growth and protein production in 96-well and 24-well plates compared to the conventional static culture method.

## Introduction

HEK293 is a preferred cell line in the biotherapeutics industry and academia. This cell line and its derivatives are notable for their quick and easy reproduction and maintenance, as well as amenability to transfection. Additionally, its human origin means it can perform most post-translational modifications. All these features have made HEK293 and its derivatives reliable resources in the biotherapeutics industry for transient and stable protein production. These cell lines have also been useful in gene therapy or cell therapy as viral vector producers for recombinant

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adenoviruses and lentiviruses.

HEK293 cells have been widely used for many years to produce industrial therapeutic proteins and research-grade proteins. Besides protein production, the HEK293 cell lines has also become a mainstay in gene therapy and cell therapy development in recent years. Together, the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have approved five therapeutic agents produced with HEK293 cells for human therapeutic use. Since 2015, the FDA has also approved six cell and gene therapies that use HEK293 and its derivatives to generate viral vectors. Additionally, academic researchers often use HEK293 cell lines to establish stably transfected cell lines to investigate signaling pathways and ligand of receptors, among other applications.

In previous application notes, we demonstrated how the C.BIRD significantly improved suspension type CHO cell line growth and protein production in 24-well plate and 96-well plate cultures. The microbioreactor's automatic agitation in each well created better circulation of nutrients in the medium, helping researchers speed up the cell line development (CLD) process for suspension cells. In this application note, we demonstrated that the C.BIRD can also improve the cell growth and protein production of adherent HEK293 in 24-well plate and 96-well plate cultures, further enhancing the research progress of this cell line.

## Materials and methods

A mAb expressing HEK293 cell line was used in this study. The cells were cultured in Minimum Essential Medium (MEM) (#10-009-CVS, Corning) with 10% Fetal Bovine Serum (#A3160601, Gibco), Penicillin-Streptomycin (#30-002-CI, Corning) and 100 µg/ mL of Hygromycin B (#10687010, Gibco) at 37°C in 5% CO<sub>2</sub> incubator. The standard 24-well plates (#662160, Greiner) and 96-well plates (#3628, Corning) were used in this experiment. Comparison studies were performed with cells cultivated in a traditional static culture as well as the novel C.BIRD mixing culture. The initial cell concentrations were divided into two groups: low initial density and high initial density. The low initial density group started from 1x10<sup>4</sup> cells/cm<sup>2</sup>, while the high initial density group started from 1x10<sup>5</sup> cells/cm<sup>2</sup>. The volume of both the static and

the C.BIRD groups was 200 µL/ well medium in the 96-well plate and 1,600 µL/ well medium in the 24-well plate. Cells in the C.BIRD mixing culture group were first cultured in static conditions for 24 hours and then applied to the C.BIRD system with continuous mixing mode at 50 seconds/cycle mixing rate. The cell culture supernatants were collected to determine the protein yields, and the cells in each group were digested and collected with 0.25% Trypsin (#25-050-CI, Corning). At indicated days, the total cell density, viable cell density and cell viability were measured in triplicate with a TC20 Automated Cell Counter (#1450102, Bio-Rad).

Protein yields were measured by IgG (Total) Human Uncoated ELISA Kit (#88-50550-88, Invitrogen). The protein fold changes were calculated by dividing the C.BIRD group protein yield by the static group protein yield. Statistics were performed by multiple unpaired t tests. Significance of P value is listed as follows: >0.05 (ns), <0.05 (\*), <0.01 (\*\*), <0.001 (\*\*\*) and <0.0001 (\*\*\*\*). Data are shown as mean ± SD.

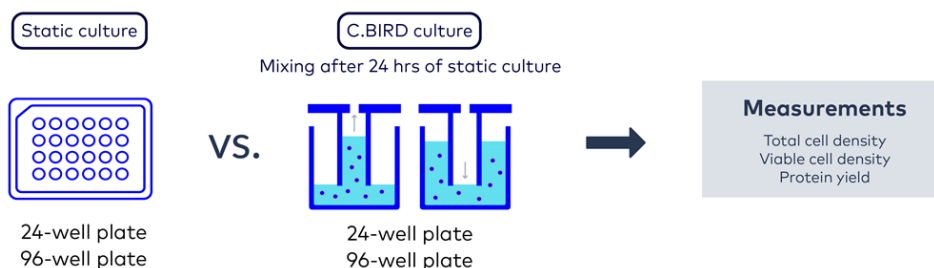


Figure 1. Diagram of experiment design.

## Results and discussion

This comparative experiment was designed as shown in **Figure 1**. Total cell density, viable cell density and protein yields of mAb expressing HEK293 cells were measured from the static culture method and the C.BIRD culture method.

### Low initial density test

The cell seeding density of the low initial test was  $1 \times 10^4$  cells/cm<sup>2</sup>. The total and viable cell densities and protein yields were measured on day 6, day 7 and day 8. In the 96-well plate test, the average total and viable cell density of the C.BIRD culture group reached  $3.29$  and  $3.12 \times 10^6$  cells/cm<sup>2</sup> on day 8, while the static culture group only reached  $1.40$  and  $1.29 \times 10^6$  cells/cm<sup>2</sup> (**Figure 2A and 2B**). The cell

viabilities of the C.BIRD group and static group were all maintained above 90% until day 8 (**Figure 2C**). The average protein yields of the C.BIRD culture group were more than two times higher than the static culture group on the measuring days (**Figure 2D**). The 24-well plate and the 96-well plate test had similar results. The average of the total and viable cell densities of the 24-well C.BIRD group were  $3.01$  and  $2.83 \times 10^6$ /cm<sup>2</sup> on day 8, nearly three times higher than the average from static culture group (**Figure 2E and 2F**). The cell viabilities of both groups also maintained above 90% until day 8 (**Figure 2G**). Additionally, the C.BIRD culture method improved the protein yields by two times compared to the static culture method in the 24-well plate scale (**Figure 2H**).

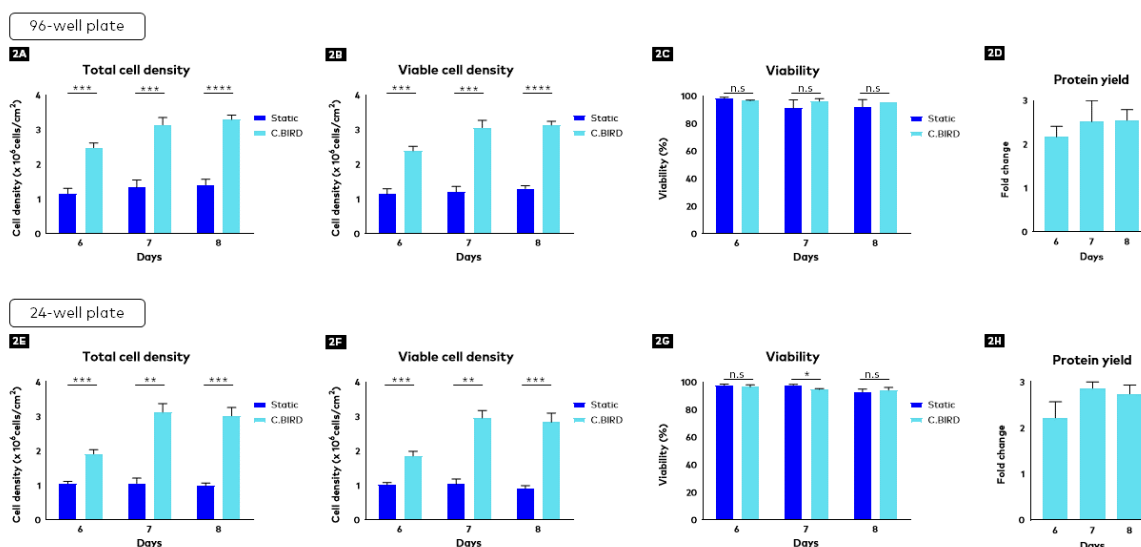


Figure 2. **A-D)** Total cell density, viable cell density, cell viability and protein yield of 96-well plate culture condition in low initial density test. **E-H)** Total cell density, viable cell density, cell viability and protein yield of 24-well plate culture condition in low initial density test. Data are shown as mean  $\pm$  SD.design

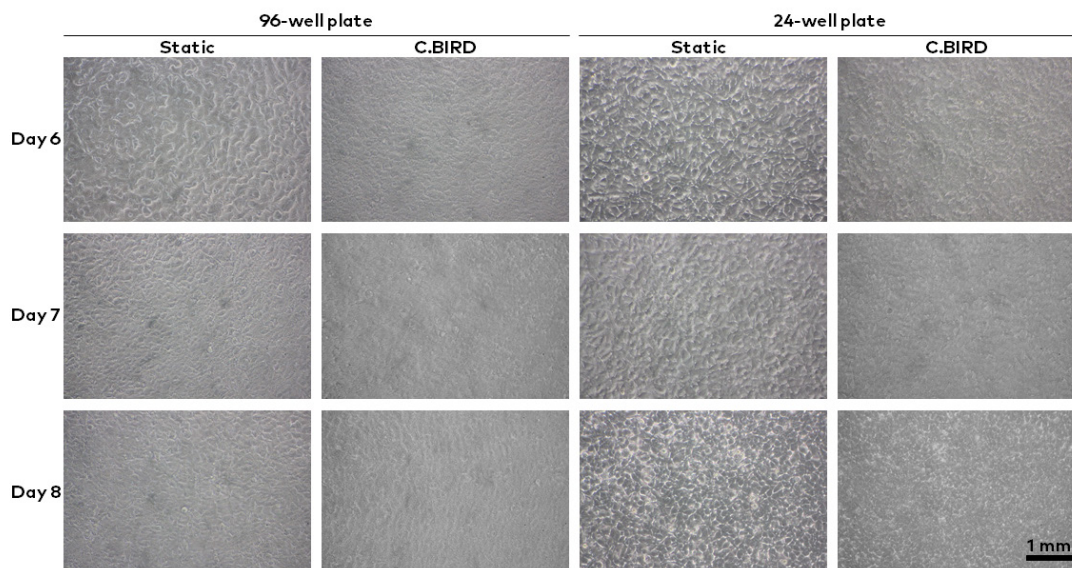
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The cell confluency of the static and C.BIRD culture methods in both 96 and 24-well plates are shown in **Figure 3**. There were hardly any dead cells found floating in either group's culture medium. However, the difference in cell density between the two groups was noticeable under microscopic observation. The cells in the C.BIRD culture group were more compact than those in the static culture group. This result was related to the cell density quantization shown in **Figure 2**.

### High initial density test

As for the high initial density test, the cell seeding density was  $1 \times 10^5$  cells/cm<sup>2</sup>. The results showed the same trend as the low initial density test. The total and viable cell densities and protein yields were measured on day 3, day 4 and day 5

(**Figure 4**). In the 96-well plate test, the average of total and viable cell density of the C.BIRD culture group reached  $3.28 \times 10^6$  cells/cm<sup>2</sup> and  $3.16 \times 10^6$  cells/cm<sup>2</sup>, which was 2.2 times higher than that of the static culture group (**Figure 4A and 4B**). The cell viabilities of both groups also maintained above 90% until day 5 (**Figure 4C**). According to the higher cell density, the protein yield of the C.BIRD culture group was nearly four times higher than the static culture group on day 5 (**Figure 4D**). As with the low initial density test, the C.BIRD culture method also improved the total and viable cell density in the 24-well plate test (**Figure 4E and 4F**). The cell viabilities were maintained above 90% in each group until day 5 (**Figure 4G**). The protein yields were significantly higher in the C.BIRD culture group than in the static culture group from day 3 to day 5 (**Figure 4H**).



*Figure 3* Bright field microscopy images of the mAb expressing HEK293 cells growth in 96-well and 24-well culture conditions of the low initial density test. The static and the C.BIRD culture groups were observed at indicated times. Scale bar = 1 mm

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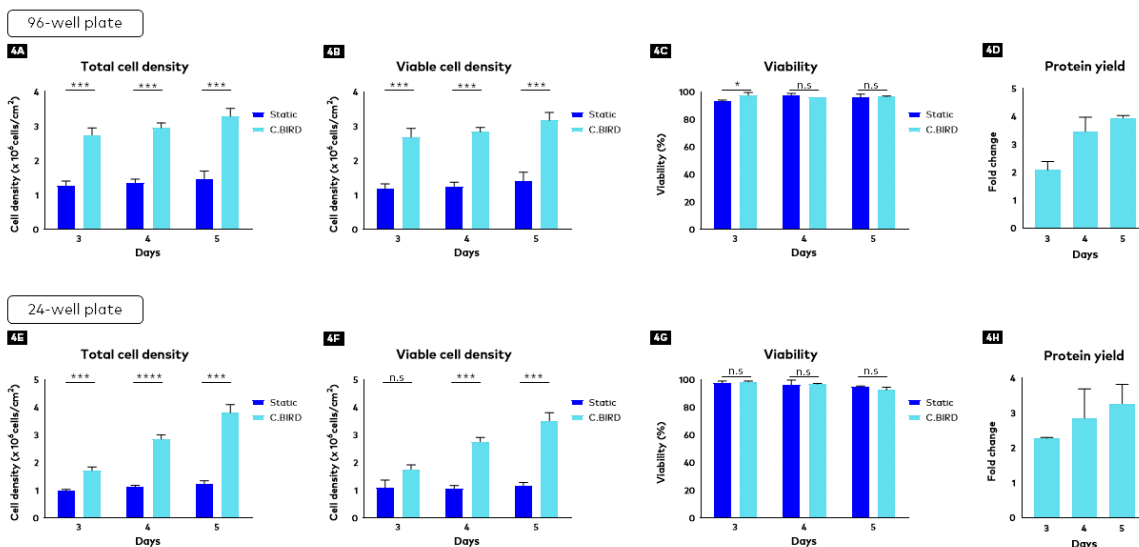


Figure 4. **A-D)** Total cell density, viable cell density, cell viability and protein yield of 96-well plate culture condition in high initial density test. **E-H)** Total cell density, viable cell density, cell viability and protein yield of 24-well plate culture condition in high initial density test. Data are shown as mean ± SD.design.

The cell confluency results in the high initial density test were similar to the results from the low initial density test. Under microscopic observation, there were few floating dead cells in the medium

of either group. Additionally, the cells appeared more compact in the C.BIRD culture group than in the static culture group (**Figure 5**).

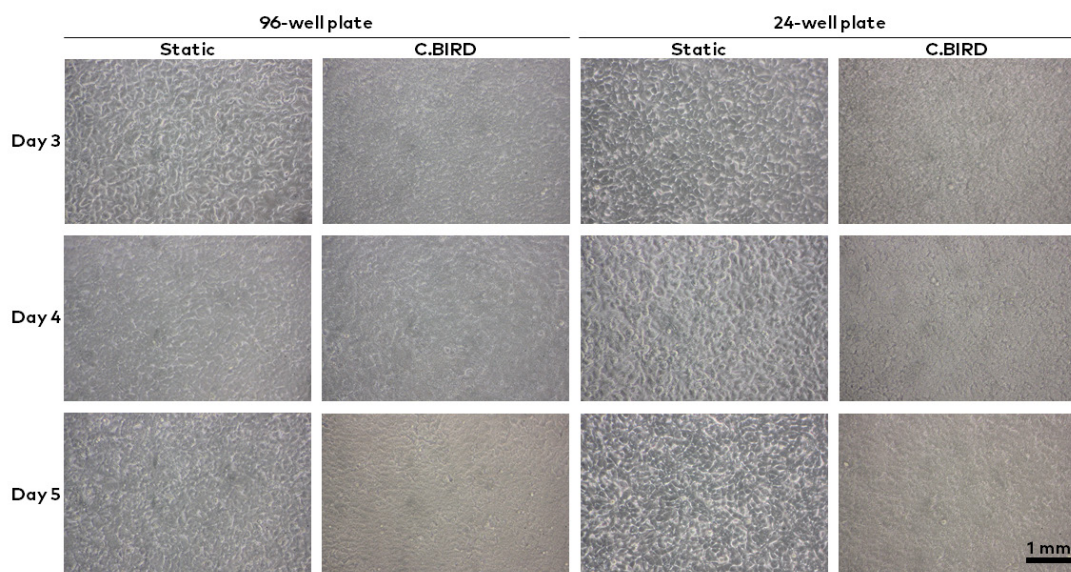


Figure 5. Bright field microscopy images of the mAb expressing HEK293 cells growth in 96-well and 24-well culture conditions of the high initial density test. The static and the C.BIRD culture groups were observed at indicated times. Scale bar = 1 mm.

## Conclusion and future direction

The HEK293 cell line and its derivatives are notable cell lines in the bioindustry. This study showed that the C.BIRD mixing culture significantly increased the cell density and protein yield of the HEK293 cell line in 96-well and 24-well plates. Furthermore, the C.BIRD microbioreactor can shorten the culture time required to obtain adequate cell numbers and protein secretion, or to increase the cell number and protein yield in a particular culture timeframe. These features can potentially speed up processes using the HEK293 cell line or its derivatives, such as biologics production, cell therapies and gene therapies.

## References

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