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# C.BIRD™ | An Innovative Cell Culture for Hybridoma in Early Stages

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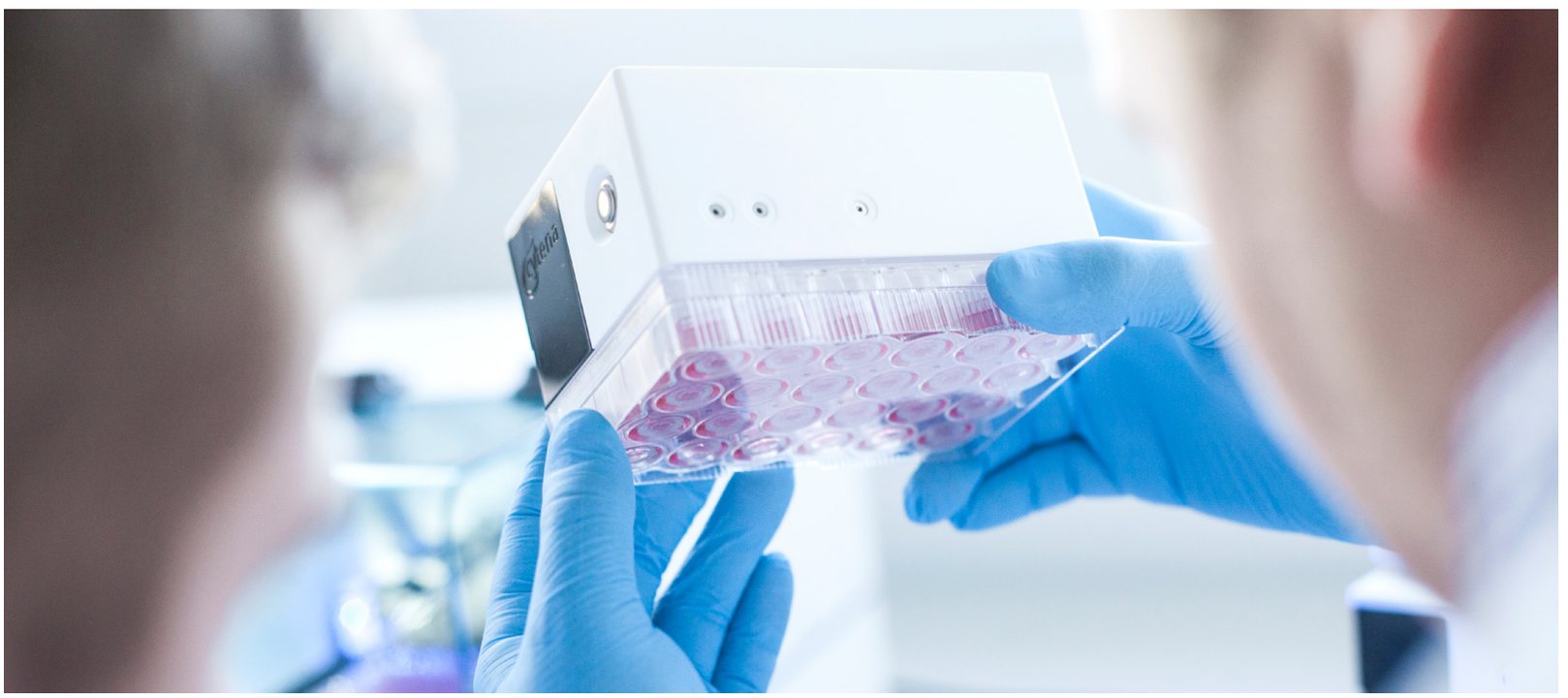

## Abstract

Hybridoma technology is the first and well-established methodology to produce monoclonal antibodies (mAbs). However, it faces the obstacles of the monoclonal cell line development, such as low growth rate and poor productivity under the low initial density. By growing single colonies in microwells, the cells endure an extended time in static culture, in which the concentration of nutrients and oxygen in the medium decreases over time. To optimize the clonal expansion from low cell density and to speed-up the antibody production for the early-stage analysis, we introduce the C.BIRD™, our innovative technology. C.BIRD™ offers continuous mixing in 96-well plates to improve the culture environment for hybridoma cells by providing a higher diffusion rate of oxygen for a more homogeneous culture environment. In this application note, we compared the cell growth and mAb production efficiencies under different culture conditions. As a result, we observed increased cell growth and significantly improved mAB production in C.BIRD™ cultures in comparison to static or mixing cultures. Our C.BIRD™ technology holds a great potential to improve the hybridoma development by shortening the timeline of culturing and early-stage analytical studies.

# Introduction

Monoclonal antibodies (mAbs) have been playing a fundamental role in biomedical industries since their discovery. The high specificity, affinity, and homogeneous nature of mAbs make them valuable in various research techniques, including flow cytometry, immunoassays, diagnostics, and also as therapeutics. During the development of mAbs, the antigen of interest is injected in a mouse and the spleen cells are isolated. After fusing the spleen cells and myeloma cells, hybridomas were distributed into microwell plates with low cell density to develop single colonies. For the humanized mAb development, mAbs should be altered to meet the requirement of human immune system in which genetically-engineered hybridomas go through another clonal selection. The time-consuming process of selecting and expanding desirable clones is one of the obstacles, slowing down the development of hybridoma workflow.

Due to the lack of nutrients and oxygen supplements during the long culture period, the poor growth rate of the cells is one of the major causes for the longdeveloping process of the technology.

In this study, we proposed a new method to distribute the nutrients and increase the oxygen diffusion in the culture medium by using the C.BIRD™. The continuous mixing of the C.BIRD™ provides a homogeneous suspension culture in microwell plates. In our result, hybridomas possessed a higher growth rate and mAb productivity by using the C.BIRD™ compared to the conventional culture methods. Undoubtedly, our method shortens the cell culture expansion process, which starts from a single colonie or a low cell density cell culture. Therefore, the C.BIRD™ has the potential to facilitate the early-stage analytic screening by shortening the required timeline for early mAb development.

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

# Materials and methods

A mouse mAb-expressing hybridoma cell line (inhouse) was used in this study. Cells were cultured in Dulbecco's Modified Eagle Medium, High glucose (#CC103-0500,GenedireX) supplemented with 10% FBS (Gibco) and 1% penicillin/streptomycin. Standard 96-well plates (#0030730011,Eppendorf) were used for C.BIRD™ and static cultures (**Figure 1**). Flasks (#431143, Corning)were placed on a shaker for mixing cultures (shaking speed: 90 rpm; orbital: 20mm). Cultures were kept at 37°C in a water-jacketed incubator with 5% $CO_2$.

The initial cell concentration was 2.5 x 103 cells/ml in all cell culture conditions. The volume of the medium in both static and the C.BIRD™ groups was 200 µl/well in standard 96-well plates, while the volume of the shaker-flask culture was 30 ml in total. Total and viable cells number and viability were counted with an automatic cell counter (TC20 from Bio-Rad) from Day 4 to Day 7. To compare the antibody production efficiencies in each cell culture method, we measured the antibody concentration using mouse IgG (Total) Uncoated ELISA kit (# 88-50400-88, Invitrogen) in the supernatants of all cultures collected at day 7. Data were analyzed by one-way analysis of variance (ANOVA). Significance of p-value is listed as the following: 0.12(ns), 0.033(*), 0.002(**), 0.0002(***) and <0.0001(****). Data are shown as mean ± SD.

# Results and discussion

The C.BIRD™ provides a perpendicular mixing in the multi-well plates which introduces a better circulation of the nutrients and oxygen by the constant flow. To compare whether the mixing of the C.BIRD™ method could improve the cell culture conditions of hybridoma, we designed the experiment as shown in **Figure 1**. We measured total cell density, viable cell density, and analyzed the viability of the three different cell culture methods. Under continuous mixing (25s/cycle) in the C.BIRD™ culture in the standard 96-well plate, the total cell density peaked at 2.44 x 106 cells/ml on average at Day 6, which were 1.99-fold and 1.89-fold higher compared to the static and shaker-flask groups, respectively (**Figure 2A**).

Additionally, viable cell density of the C.BIRD™ group an average of 2.04 x 106 cells/ml at Day 6 and the other two groups had only an average of 9.22 x 105 cells/ml and 1.11 x 106 cells/ml in static and shaker-flask groups, respectively (**Figure 2B**). The results showed significant improvement in cell growth in the C.BIRD™ cultures. C.BIRD™ provided more efficient conditions to expand the hybridoma cells from a relatively low cell density.

On the other hand, the cell viability in the C.BIRD™ group was not compromised, regardless of the high cell density (**Figure 2C**). On Day 6, while sustaining the highest viable cell density within three groups, the C.BIRD™ group had 83% of viability on average, close to that of the shaker-flask group (87%) and was higher than the static group (75%). The result demonstrated that the C.BIRD™ method could increase environmental carrying capability and helped to expand the cell population. To investigate the efficiency of Mouse mAb production, supernatants were collected at Day 7. We measured a high concentration of mAb in the C.BIRD™ group, which reached an average of 3309.2 µg/mL, and outcompeted that in both static and shaker-flask groups (**Figure 2D**).

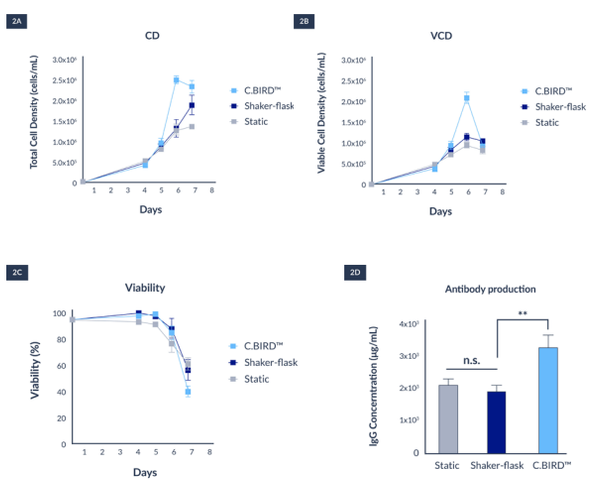


***Figure 2.*** *The cell growth and antibody production of three culture methods in 7 days of continuous culture. he C.BIRD facilitated the cell growth in both* ***A)*** *total cell density and* ***B)*** *viable cell density while maintaining the same* ***C)*** *viability as the other two groups. In addition, the C.BIRD group showed a significantly higher* ***D)*** *antibody production at Day 7 compared to both static and shaker-flask groups.*

# Conclusion

Hybridoma cultures share similar issues with low productivity and cell density, the C.BIRD™ method provided an innovative way to boost cell growth in an extremely low seeding cell density. Our study showed a magnificent improvement in 1) total cell density, 2) viable cell density, 3) maintenance of viability during long-term culture, and 4) high productivity of mAb, comparing to static culture in 200 µL/well conditions. More excitingly, the C.BIRD™ group outperformed the shaker-flask group in cell growth and antibody production. By using the C.BIRD™, researchers can accelerate clonal selection and shorten the timeline for hybridoma development.

# Reference

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