Efficient Expansion of HEK-293 Suspension Cell Cultures in Corning® 5L Erlenmeyer Flasks

Application Note

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Introduction

HEK-293F is a variant of the HEK-293 cell line that has been adapted for suspension growth. As the HEK-293F expression system permits the transfection of large volumes of cells, demonstrates high transfection efficiencies, and eliminates the need for changing media, many laboratories use this suspension cell culture system for generating large amounts of mammalian recombinant proteins.

In this study, we assessed the performance of Corning 5L Erlenmeyer flasks with different media fill volumes on the growth of HEK-293F cells. Three different working culture volumes were tested for each flask type (2.5L, 3L, 3.5L). We also performed a benchmark study using HEK-293F cells to compare the cell yields and viabilities of culture conditions in Corning 5L Erlenmeyer flasks and a Comparable baffled bottom 5L Erlenmeyer flask.

Corning disposable Erlenmeyer flasks are ideal for the storage and applications of shaker cultures. The Corning 5L Erlenmeyer flask features a large vent cap that enables the scale-up of suspension cell cultures by providing continuous gas exchange while ensuring sterility and preventing leaks. Corning 5L Erlenmeyer flasks are also space-efficient and provide an ideal choice for culturing large quantities of cells. These flasks are available in PC or PETG with plain bottom or baffled bottom designs, thus offering different choices according to specific cell lines.

Materials and Methods

Cell culture conditions

FreeStyle™ 293-F cells (Thermo Fisher R79007) were acclimated to suspension culture in defined, serum-free FreeStyle 293 expression medium (Thermo Fisher 12338026), as recommended. The cells were cryopreserved in a vial containing 1 mL of cells at a density of 1 x 10^7 viable cells/mL in 90% FreeStyle 293 expression medium and 10% DMSO and stored in liquid nitrogen until further use. The cryogenic vial of cells was removed from the liquid nitrogen and thawed quickly in a water bath at 37°C. The contents of the cryogenic vial were transferred into a 125 mL Corning Erlenmeyer flask (Corning 431143) and supplemented with 19 mL of pre-warmed FreeStyle 293 expression medium. The cells were incubated in a 37°C shaker incubator containing a humidified atmosphere of 8% CO_2 in air, with agitation at 110 rpm.

Cell Expansion

For the general maintenance of HEK-293F cells, the cells were passaged at a seeding density of 5 x 10^5 cells/mL when they reached a density of approximately 2 x 10^6 viable cells/mL (generally every 3 to 4 days). Log-phase cultures should be >90% viable. The trypan blue exclusion assay was used to determine cell viability. The cells were sequentially passaged at a seeding density of 2 x 10^5 cells/mL from a Corning 125 mL Erlenmeyer flask (Corning 431143) to Corning 1L Erlenmeyer flasks (Corning 431147), and finally to Corning and Comparable 5L Erlenmeyer flasks.

A shaker incubator was used at an agitation speed of 90 rpm for cell culture in 125 mL and 1L Erlenmeyer flasks, and at an agitation speed of 110 rpm for cell culture in 5L Erlenmeyer flasks.

Benchmarking

FreeStyle 293-F cells cultured in 5L Erlenmeyer flasks were finally seeded at a density of 2 x 10^5 cells/mL into 4 different flasks: A Corning PC 5L baffled bottom Erlenmeyer flask (Corning 431684), a Corning PETG 5L baffled bottom Erlenmeyer flask (Corning 431285), a Corning PETG 5L plain Erlenmeyer flask (Corning 431284), and a Comparable PP 5L Erlenmeyer flask. Three different media fill volumes (2.5L, 3L, 3.5L) were tested. The cell densities and cell viabilities were measured every two days for eight days.

Results

As shown in Figure 1, when the 5L Erlenmeyer flasks were filled with a working volume of 2.5L of the culture medium, the performance of our Corning flasks and the Comparable flask was similar. Comparable growth rates and viabilities of HEK-293F cells were observed in all flasks, regardless of the four different growth conditions.

As shown in Figure 2, when the working volume was increased to 3L, the performance of the Corning 5L Erlenmeyer flasks is approximately the same as that of the working volume of 2.5L, regardless of the material and shape of the flasks. However, when the culture volume was 3L, the viable cell density and viability of the Comparable flask was lower than the Corning flasks. This observed difference in performance might be due to the Corning 5L flasks having a larger absolute total volume than the Comparable flask.

When the fill volume of the medium was increased to 3.5L, our results show there was no significant difference in the viable cell density and viability of HEK-293F cells when they were cultured at fill volumes of 2.5L and 3L (Figure 3). However, the performance of the Comparable's 5L Erlenmeyer flask at the 3.5L fill volume was not as efficient as that of the Corning® 5L Erlenmeyer flasks. This may be due to the absolute total volume of the Comparable 5L Erlenmeyer flask being less than that of the Corning 5L Erlenmeyer flasks, leading to poor gas exchange in the former.

Since cell density and cell viability remain close when the fill volume of the medium was increased to 3.5L, we received more viable cell yield at 3.5L fill volume than 2.5L and 3L. We calculated total cell yield via multiplying density of viable cells by volume. Figure 4 shows the total cell yield of HEK-293F at day 8 with three different fill volumes. If the volume is increased from 2.5L to 3.5L, more cells can be achieved in Corning 5L Erlenmeyer flasks without decreasing the viability.

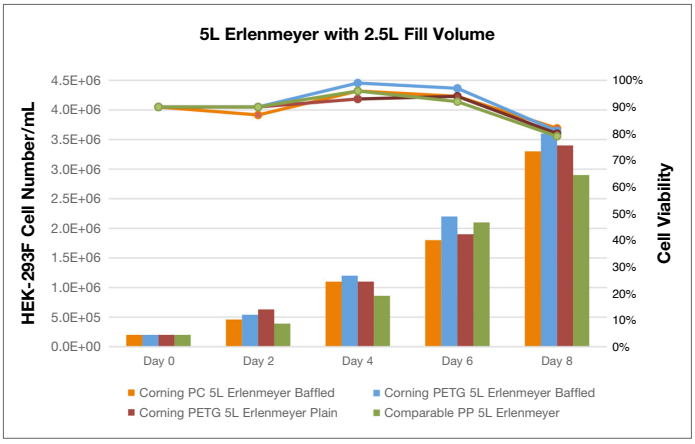

Figure 1. Viable cell density and viability of HEK-293F cells cultured in a Corning PC 5L Erlenmeyer flask (baffled), Corning PETG 5L Erlenmeyer flask (baffled), Corning PETG 5L Erlenmeyer flask (plain), and a Comparable PP 5L Erlenmeyer flask at a fill volume of 2.5L. The data were measured every 2 days for 8 consecutive days.

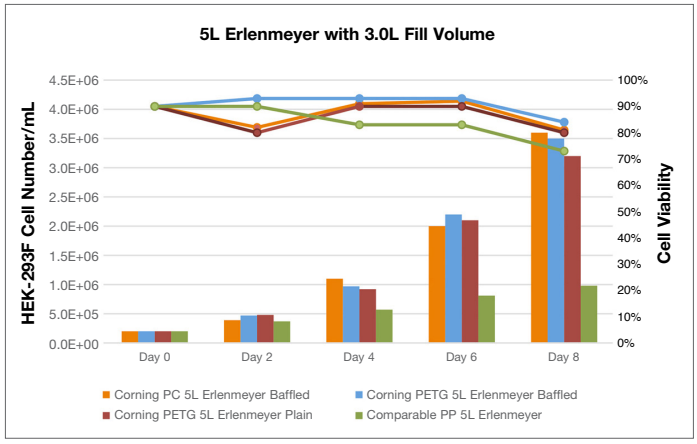

Figure 2. Viable cell density and viability of HEK-293F cells cultured in a Corning PC 5L Erlenmeyer flask (baffled), Corning PETG 5L Erlenmeyer flask (baffled), Corning PETG 5L Erlenmeyer flask (plain), and a Comparable PP 5L Erlenmeyer flask at a fill volume of 3L. The data were measured every 2 days for 8 consecutive days.

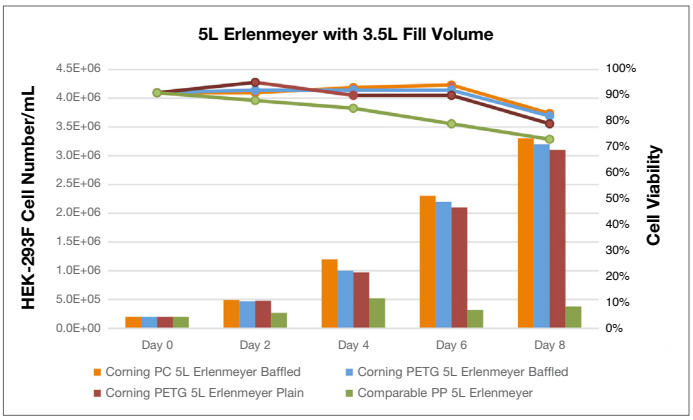

Figure 3. Viable cell density and viability of HEK-293F cells cultured in a Corning PC 5L Erlenmeyer flask (baffled), Corning PETG 5L Erlenmeyer flask (baffled), Corning PETG 5L Erlenmeyer flask (plain), and a Comparable PP 5L Erlenmeyer flask at a fill volume of 3.5L. The data were measured every 2 days for 8 consecutive days.

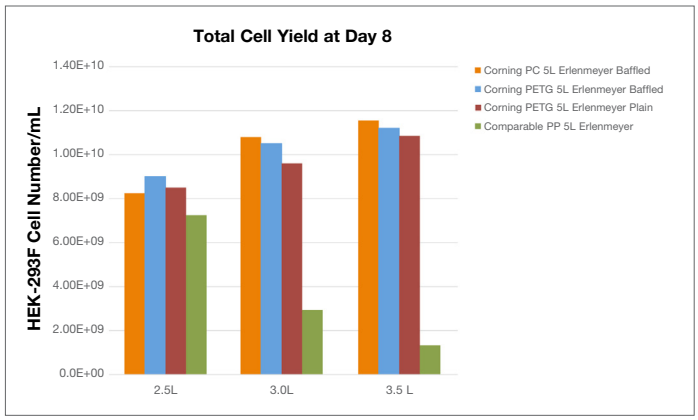

Figure 4. Total cell yield at day 8 in HEK-293F cells cultured in a Corning PC 5L Erlenmeyer flask (baffled), Corning PETG 5L Erlenmeyer flask (baffled), Corning PETG 5L Erlenmeyer flask (plain), Corning PETG 5L Erlenmeyer flask, and a Comparable PP 5L Erlenmeyer flask at three different fill volumes.

Conclusions

- All three Corning® 5L Erlenmeyer flasks (Corning PC 5L baffled bottom, Corning PETG 5L baffled bottom, and Corning PETG 5L plain bottom) support the efficient growth and high viability of HEK-293F cells.
- Corning 5L Erlenmeyer flasks show approximately the same performance with regards to viable cell density and viability at different fill volumes (2.5L, 3L, 3.5L). Increasing the fill volume to 3.5L does not degrade the performance of these flasks.
- Comparable growth rates and viabilities of HEK-293F cells were observed among the Corning 5L Erlenmeyer flask and the Comparable 5L Erlenmeyer flasks when the fill volume of the medium was 2.5L. However, a significantly higher viable cell density was seen in the Corning 5L flasks than in the Comparable flask at culture volumes of 3L and 3.5L.
- As the volume can be increased from 2.5L to 3.5L while keeping the performance consistent, the total viable cell number/flask can be increased. This makes it possible to reduce the cost of consumables and labor for the scale-up of HEK-293F suspension cell culture systems and generate large amounts of recombinant proteins.

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