

Corning® PF Growth Medium for Protein-free Expansion of CHO-S and AE-1 Cells

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Application Note

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Introduction

Fetal bovine serum (FBS) is traditionally used as a supplement in media for cell and tissue culture at final concentrations of 5% to 10%. For many cell lines, it is essential for cell growth when using classical media formulations. However, the undefined combination of proteins, hormones, and growth factors that render FBS so effective in promoting cell attachment and growth, is precisely the source of the problems with serum supplementation. The high protein content of serum can introduce complications with protein purification.¹ As an undefined biological product, there is significant lot-to-lot variability, which may negatively impact cellular and molecular studies. Lot-to-lot variation can introduce artifacts due to the presence of unknown compounds, proteins, growth factors, and other undefined components.² For these reasons, the use of serum can lead to downstream complications when used in biomanufacturing. Consequently, there is a movement to reduce bovine serum in developing human therapeutics.

Many are advocating for serum and protein replacement altogether – given the list of disadvantages associated with FBS supplementation – for both routine cell culture and for cell culture oriented towards clinical applications.²⁻⁴ Thus, formulated and low-protein alternatives are of increasing value. Accordingly, Corning has several options including Corning PF growth medium, which is a proprietary serum-free and protein-free growth medium containing no hormones, growth factors, or L-glutamine. The following study highlights the advantages of Corning PF growth medium for culture of suspension-adapted Chinese Hamster Ovary (CHO-S) and suspension-adapted mouse hybridoma (AE-1) cells – common cell lines for biotherapeutic protein and antibody production, respectively. When used as a direct replacement for the recommended protein-free media, both CHO-S and AE-1 cells cultured in Corning PF growth medium adapted quickly to the new conditions with consistent doubling times and higher cell viability.

Materials and Methods

CHO-S Suspension Culture

CHO-S cells (Thermo Fisher Cat. No. A1155701) were thawed according to manufacturer's recommendations. Evaluation of Corning PF growth medium (Corning Cat. No. 40-200-CV) versus Competitor A and Competitor B protein-free media was initiated in 125 mL disposable spinner flasks (Corning Cat. No. 3152) at

post-thaw passage 10 to 20. All 3 of the media were supplemented with 8 mM L-glutamine (Corning Cat. No. 25-005-Cl). For each study, three 125 mL disposable spinner flasks were seeded with CHO-S cells at 1×10^5 cells/mL (40 mL/vessel) in each medium. Cells were cultured for 72 to 96 hours on top of a 4-place slow speed magnetic stirrer at 60 rpm in a humidified 37°C/5% CO₂ cell culture incubator. Cells were harvested after 72 or 96 hours, regardless of the cell density. Cells were briefly mixed to obtain a homogenous mixture before sampling for cell count and viability. Each vessel was sampled for cell enumeration and viability measurements using a ChemoMetec NucleoCounter® NC-200™ automated cell counter. The remaining cell suspensions for each medium type were pooled and centrifuged at $250 \times g$ for 5 minutes. The supernatant was aspirated and cells were resuspended in their appropriate medium to do a final count for seeding into 3 new vessels per condition for subsequent passaging. Each study continued for 5 sequential passages and was repeated, in full, 3 independent times. At each passage, the cell count was determined and was used to calculate the doubling time and the total mean cell yield.

AE-1 Suspension Culture

AE-1 cells (ATCC® HB-72™) were thawed according to manufacturer's recommendations. Evaluation of Corning PF growth medium versus Dulbecco's Modified Eagle's Medium (DMEM; Corning Cat. No. 10-013-CM) supplemented with 10% FBS (Corning Cat. No. 35-015-CV) and a Competitor protein-free media was initiated in T-75 cell culture flasks (Corning Cat. No. 430641U) at post-thaw passages 5 through 15. Corning PF medium was supplemented with 4 mM L-glutamine, whereas the other media were already formulated with L-glutamine. For each study, 3 T-75 cell culture flasks were seeded with AE-1 cells at 1×10^5 cells/mL (15 mL/vessel) for each medium. Cells were cultured for 72 to 96 hours in a humidified 37°C/5% CO₂ cell culture incubator. Cells were harvested and counted as above after 72 or 96 hours, independent of cell density at the time of harvest. After centrifugation to pellet the cells, a fraction of the supernatant was collected for antibody quantification via the Easy-Titer™ Mouse IgG Assay Kit (Thermo Fisher Cat. No. 23300) following the manufacturer's recommended protocol. Each study continued for 5 sequential passages and was repeated, in full, 2 independent times. Cell count determined at each passage was used to calculate doubling time at each passage and total mean cell yield.

Results

CHO-S Suspension Culture

To assess the ability of Corning® PF growth medium to directly replace the standard protein-free media for CHO-S cell culture, the performance of Corning PF growth medium was compared to two PF media, Competitor A, which was recommended, and Competitor B. With regular subculture in Corning PF growth medium, there was a significantly higher total mean yield for 5 passages than in Competitors A and B ($1 \times 10^8 \pm 0.2 \times 10^8$ cells, $0.7 \times 10^8 \pm 0.2 \times 10^8$ cells, and $0.8 \times 10^8 \pm 0.2 \times 10^8$ cells, respectively; Figure 1). Cells cultured in all media displayed high viability (>96%) over the course of 5 passages (Figure 2), with a small but significant decrease in viability of cells grown in Competitor B at passage 4 and in Competitor A at passage 5. Future study would be necessary to determine if this trend continued with subsequent subculture. Importantly, similar doubling times were observed for cells grown in Corning PF growth medium versus the competitor media, with significantly faster doubling at passages 1 and 5 (Figure 3).

AE-1 Suspension Culture

To assess the ability of Corning PF growth medium to directly replace the recommended DMEM supplemented with 10% FBS for AE-1 cell culture, the performance of Corning PF growth medium was compared to a Competitor protein-free medium. Routine passage of AE-1 cells in Corning PF medium yielded a significantly higher total mean yield for 5 passages than either DMEM supplemented with 10% FBS or Competitor medium ($2 \times 10^7 \pm 0.6 \times 10^8$ cells $2 \times 10^7 \pm 0.3 \times 10^7$ cells, and $1 \times 10^7 \pm 0.3 \times 10^7$ cells, respectively; Figure 4). Cells cultured in all media displayed high viability (>92%) over the course of 5 passages, with no significant differences between media across all passages (Figure 5). Similar doubling times were observed for cells grown in Corning PF growth medium and the recommended DMEM supplemented with 10%

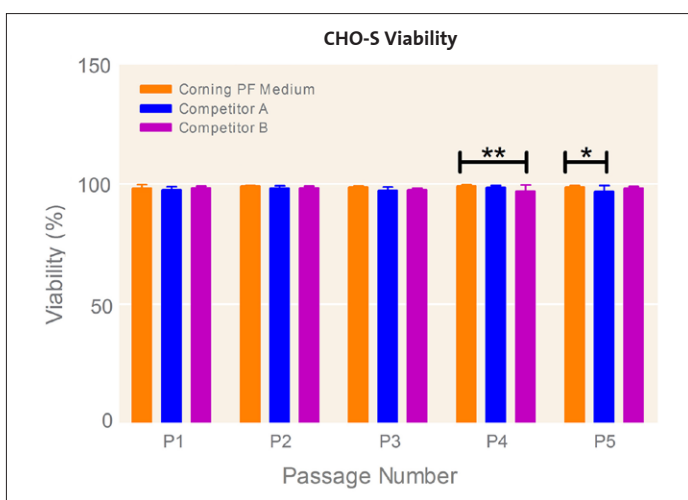


Figure 2. Viability of CHO-S cells maintained in Corning PF Medium. CHO-S cells, regardless of whether cultured in Corning PF medium, Competitor A, or Competitor B media, displayed similar cell viability over the course of the first 3 passages. Average viability of >96% was maintained in each culture across 5 passages with significantly lower viability of Competitor media at passages 4 and 5. Data shown are mean \pm SD. * $p < 0.05$ and ** $p < 0.01$ in two-way ANOVA with Bonferroni's post-test; $n = 9$ samples at each passage.

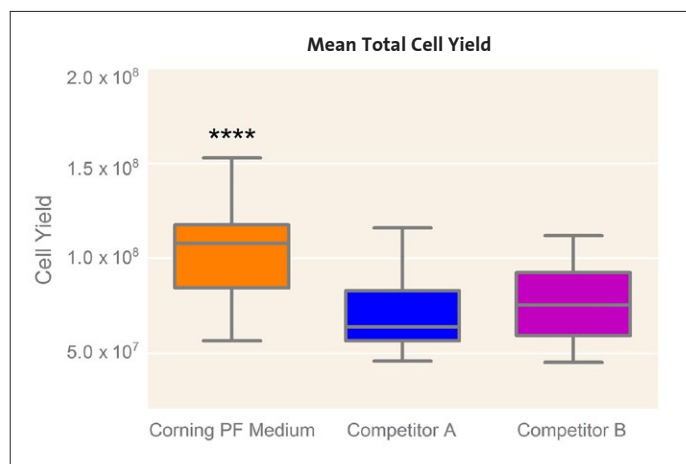


Figure 1. Higher total yield of CHO-S cells in Corning® PF Medium. As an average across 5 passages of CHO-S cells cultured in Corning PF medium, Competitor A, and Competitor B media, the total mean yield was significantly higher in Corning PF medium. The solid line marks the mean. Whiskers mark the minimum and maximum values. Lower and upper box limits mark the 25th and 75th quartile, respectively. **** $p < 0.0001$ in one-way ANOVA with Tukey's post-test; $n = 30$ samples from 2 replicates of 5 passages.

FBS. In addition, AE-1 cells cultured in Corning PF medium exhibited significantly faster doubling times at passages 1 through 4 than cells grown in the Competitor medium, indicative of more favorable cell proliferation conditions (Figure 6). Notably, this active cell proliferation in Corning PF medium supports antibody production, as measured by supernatant IgG. IgG levels in supernatant from cultures in the recommended DMEM supplemented with 10% FBS were maintained in Corning PF medium. Though not significant, the Competitor trended toward lower levels of IgG (Figure 7).

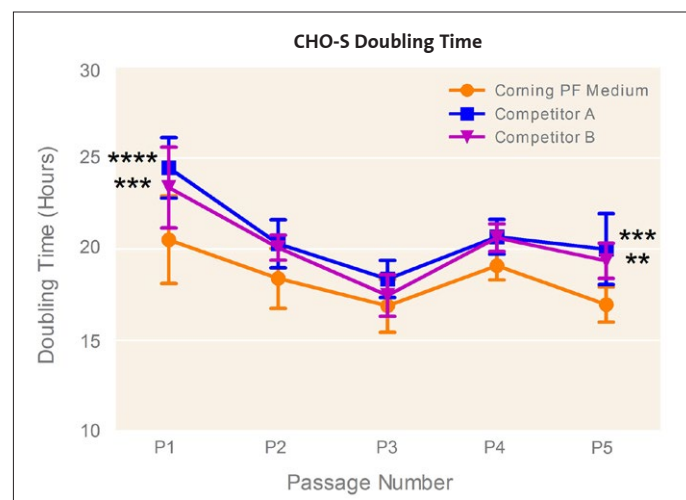


Figure 3. Consistent doubling time of CHO-S cells in Corning PF Medium. CHO-S cells cultured in Corning PF medium exhibited similar doubling times to cells in the Competitor A and Competitor B media, with significantly faster doubling times at passages 1 and 5 compared to both competitors. Data shown are mean \pm SD. ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ in two-way ANOVA with Bonferroni's post-test; $n = 9$ samples at each passage.

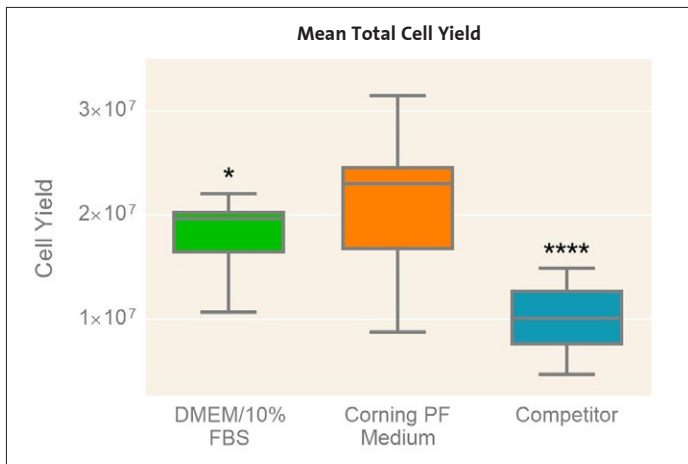


Figure 4. Higher total yield of AE-1 cells in Corning PF Medium. As an average across 5 passages of AE-1 cells cultured in DMEM plus 10% FBS, Corning PF medium, and Competitor's media, the total mean yield was significantly higher in Corning PF medium. The solid line marks the mean. Whiskers mark the minimum and maximum values. Lower and upper box limits mark the 25th and 75th quartile, respectively. * $p < 0.05$ and **** $p < 0.0001$ relative to Corning PF medium in one-way ANOVA with Tukey's post-test; $n = 30$ samples from 2 replicates of 5 passages.

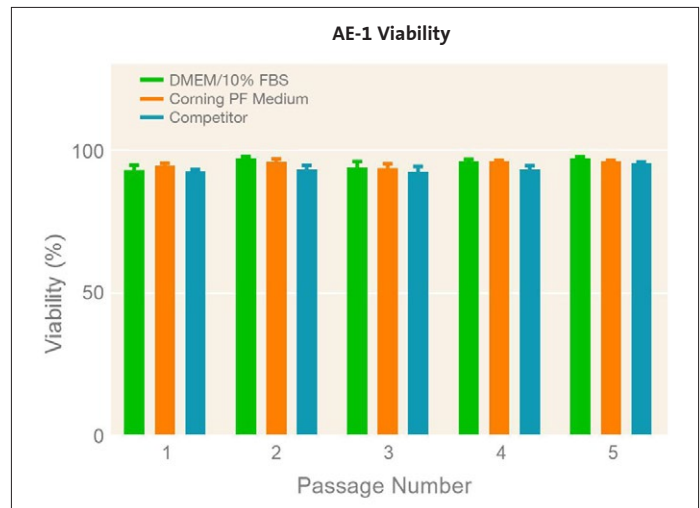


Figure 5. Viability of AE-1 cells maintained in Corning PF Medium. AE-1 cells displayed similar cell viability over the course of the 5 passages, regardless of the media. Average viability of $>92\%$ was maintained in each culture across 5 passages. Data shown is mean \pm SD; $n = 6$ samples at each passage.

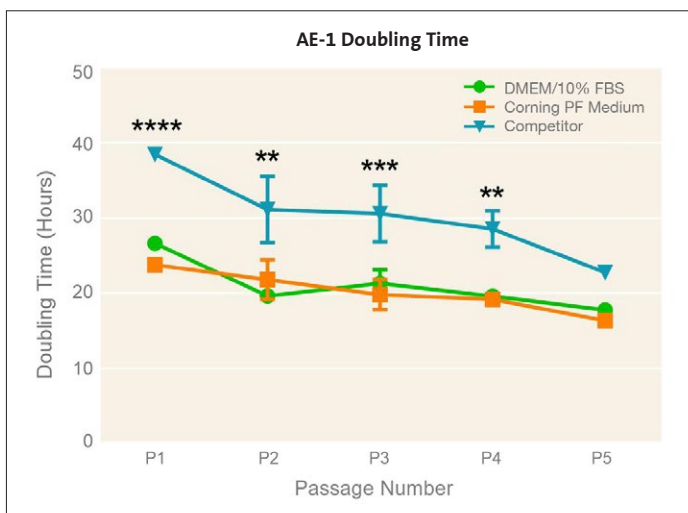


Figure 6. Consistent doubling time of AE-1 cells in Corning PF Medium. AE-1 cells cultured in Corning PF medium exhibited similar doubling times to cells in the vendor recommended DMEM plus 10% FBS. Cells cultured in Competitor's media exhibited significantly slower doubling times at passages 1 through 5. Data shown is mean \pm SD. ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ relative to Corning PF medium in two-way ANOVA with Bonferroni's post-test; $n = 6$ samples at each passage.

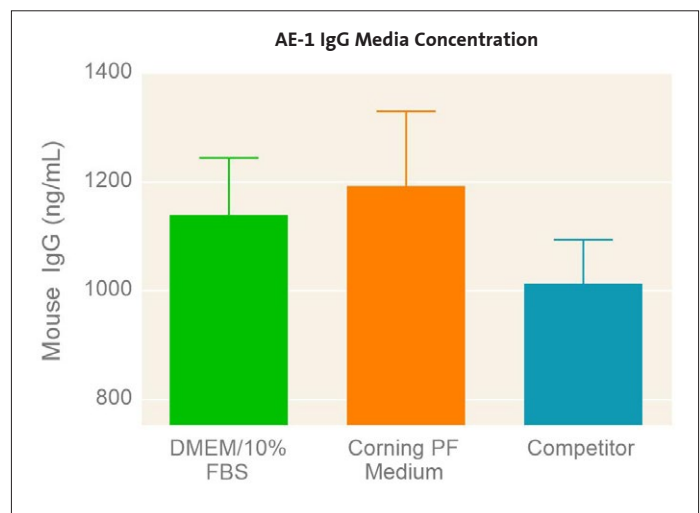


Figure 7. Antibody production levels maintained in Corning PF medium. Samples of media supernatants were collected at the time of each passage. IgG levels were measured with the Easy-Titer™ Mouse IgG Assay Kit (Thermo Fisher).

Conclusion

The higher yield and comparable, if not shorter, cell doubling time supports the use of Corning® PF growth medium as a direct replacement for the vendors' recommendations for maintenance and expansion of both suspension cells, such as the CHO-S and AE-1 utilized in this study.

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