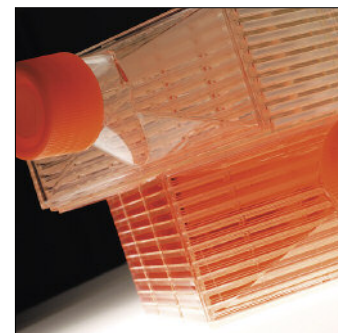


# Large-Scale Adherent Cell Protein Production Using the Corning® HYPERFlask® Cell Culture Vessel



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## Abstract

The mass-production of high-quality proteins and other biologicals is crucial to basic research as well as drug discovery. The Corning High Yielding Performance *Flask* (HYPERFlask) cell culture vessel was introduced to meet this demand. With the same overall dimensions as a conventional T175 flask but with approximately ten times the total growth surface generated by ten individual, gas permeable layers, the HYPERFlask vessel serves as an ideal option for maximizing cell yield and protein production while minimizing investment. This study demonstrates that adherent cells, modeled by the Chinese hamster ovary (CHO) 5/9 alpha cell line, reach equal densities in the HYPERFlask vessel as compared to a standard T-175 flask, with total cell yield nine times greater in the HYPERFlask vessel. Importantly, results showed greater protein production per cell in the HYPERFlask vessel, resulting in greater than tenfold total protein yields. This suggests better performance of cells grown in the HYPERFlask vessel versus conventional T-flasks.

## Methods and Materials

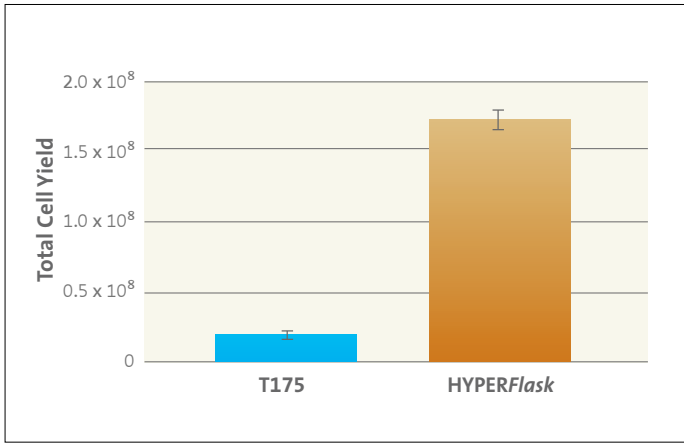
5/9 m alpha 3-18 CHO cells (ATCC® No. 10154 CRL™) were seeded into T175 flasks (Corning Cat. No. 431306) and HYPERFlask vessels (Corning Cat. No. 10010) at a density of  $5 \times 10^4$  cells/mL in 0.29 mL/cm<sup>2</sup> of IMDM (Mediatech Cat. No. 10-016-CV) supplemented with 10% Fetal Bovine Serum (FBS; Mediatech Cat. No. 35-010-CV). The vessels were placed in a humidified incubator at 37°C and 5% CO<sub>2</sub> for 96 hours. At the end of the 96-hour growth period, one milliliter samples were collected from each flask and centrifuged at 270 RCF for 7 minutes. The supernatant was collected and used to determine human macrophage colony stimulating factor (M-CSF) production following the R & D Systems Quantikine ELISA kit protocol (Cat. No.

DMC00). Each vessel was then harvested and viable cells were counted using the Nova BioProfile Flex via trypan blue exclusion. The experiment was replicated four times, each performed in triplicate.

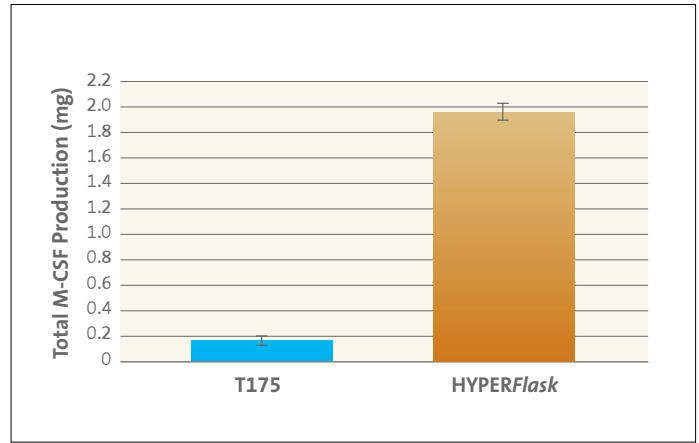
## Results

The 5/9 m alpha 3-18 CHO cell line, a commercially available CHO cell clone that constitutively expresses human macrophage colony stimulating factor (M-CSF), was selected to demonstrate adherent cell growth and protein production in the HYPERFlask vessel as compared to a standard T175 flask. Results after 96 hours showed a total cell yield nine times greater in the HYPERFlask vessel as compared to a traditional T175 flask (Figure 1), with statistically equivalent cell densities (in cells/cm<sup>2</sup>) achieved in both vessels (data not shown). As another measure of performance of the HYPERFlask vessel we measured the production of constitutively expressed M-CSF. Interestingly, total M-CSF yield was 11-fold greater in the HYPERFlask vessel compared to that of a T175 (Figure 2). Determining the amount of M-CSF production per cell demonstrated the production in the HYPERFlask vessel was 1.24 times greater than that of a T175 flask (Figure 3). These results are similar to results recently shown for antibody production from suspension hybridoma cells ([www.corning.com/lifesciences/HYPERFlask\\_suspension](http://www.corning.com/lifesciences/HYPERFlask_suspension)). Although the exact mechanism for this is unknown, this does suggest enhanced cell performance and an increased capacity for protein production in the HYPERFlask vessel. This increased production may stem from the growth of the cells on the gas permeable layer which results in the cells being in contact with oxygen molecules as they move from the external environment into the flask through the film.

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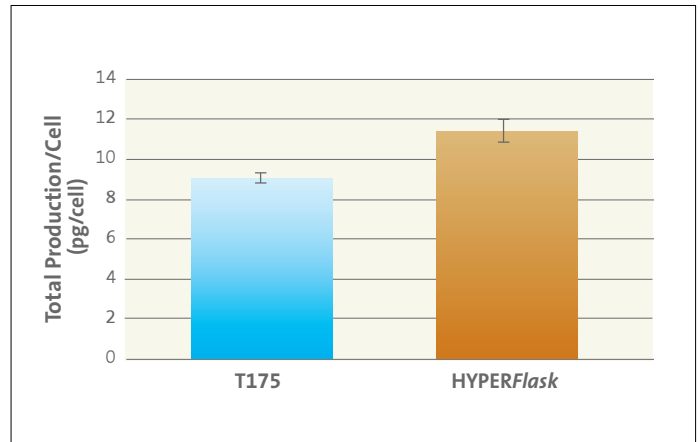
**Figure 1.** Total 5/9 alpha cell yield after 96 hours in T175 and HYPERFlask® cell culture vessels. Data represent an average of four independent studies each done in triplicate. (±S.E. indicated by error bars).



**Figure 2.** Total M-CSF production after 96 hours in T175 and HYPERFlask cell culture vessels. Data represent an average of four independent studies each done in triplicate (±S.E. indicated by error bars).

### Conclusions

- ▶ Total adherent cells yields are nine times greater in the HYPERFlask vessel versus a standard Corning® T175 flask.
- ▶ Total M-CSF production in the HYPERFlask vessel is 11-fold greater than that of a T175 flask.
- ▶ Average M-CSF production per cell is 1.24 times greater for 5/9 alpha CHO cells grown in the HYPERFlask vessel, indicating improved cell performance in the HYPERFlask vessel in comparison to conventional T-flasks.



**Figure 3.** Average M-CSF production/cell (pg/cell) after 96 hours in T175 and HYPERFlask cell culture vessels. Data represent an average of four independent studies each done in triplicate (±S.E. indicated by error bars; p = .0058).

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