

Corning® HYPERStack® Cell Culture Vessel: Performance Analysis

Application Note

CORNING

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Introduction

Production quantities of recombinant proteins, antibodies, viruses, vaccines, and cells are commonly required by researchers and manufacturers in biotechnology and pharmaceutical industries. Corning has a line of closed system modular vessels designed for growing adherent cells in large quantities. The Corning HYPERStack cell culture vessel features Corning's HYPER (High Yield PERFORMANCE) technology. The proprietary HYPER technology, using gas permeable film, eliminates the air gap within the vessel resulting in an increased cell growth surface area compared to traditional cell culture vessels. The first example of this technology was demonstrated with the Corning HYPERFlask® cell culture vessel, which utilized the HYPER technology to provide 1,720 cm² cell growth surface area (10 layers) in the same spatial footprint as a T-175 vessel. The HYPERStack cell culture vessel line is available in approximately the same spatial footprint as the 2-, 10-, and 40-layer traditional stacked cell culture vessels exhibited by the Corning CellSTACK® cell culture chambers or the Nunc® Cell Factories. Each HYPERStack module is composed of 12 individual chambers featuring the Corning CellBIND® surface treatment for

optimal cell attachment. An individual module provides 6,000 cm² cell growth surface area. The modules are joined together to form the HYPERStack 36-layer vessel (18,000 cm²) (Figure 1). The closed system, compact design, and optimized performance of the HYPERStack vessel make it ideal for use in large-scale production of cells, vaccines, and protein therapeutics.

To demonstrate the capabilities of the HYPERStack vessel, several commonly used cell lines were assessed for growth, viability, and morphologic health. In addition, the cell growth environment was analyzed by monitoring dissolved gases, electrolytes, nutrients, and cellular metabolites. Analysis of both CO₂-dependent and CO₂-independent growth environments were investigated to determine the utility of the HYPERStack vessel under different growth conditions. Recovery from cryogenic storage and cell function assessment using the label-free Corning Epic® technology were also measured for cells derived from HYPERStack vessels. Finally, crystal violet staining confirmed there was uniform cell distribution, adherence, and upon harvesting, complete cell recovery from vessels.

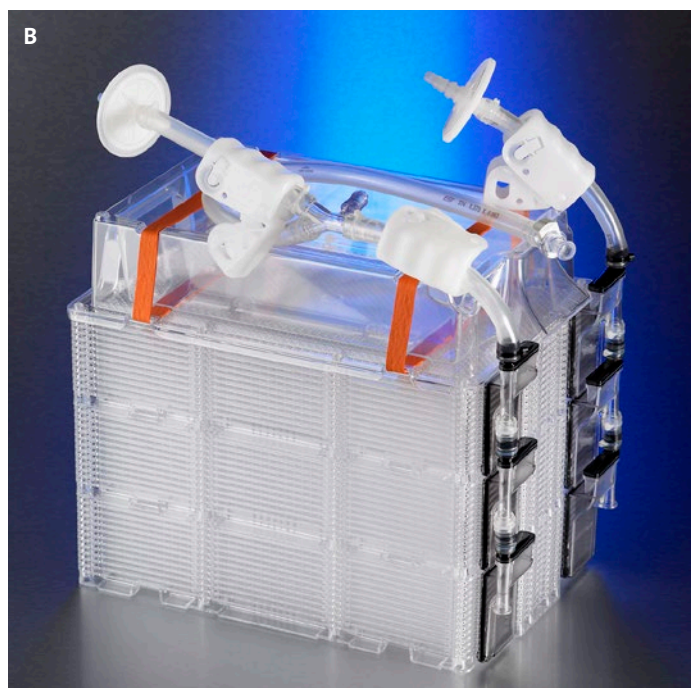
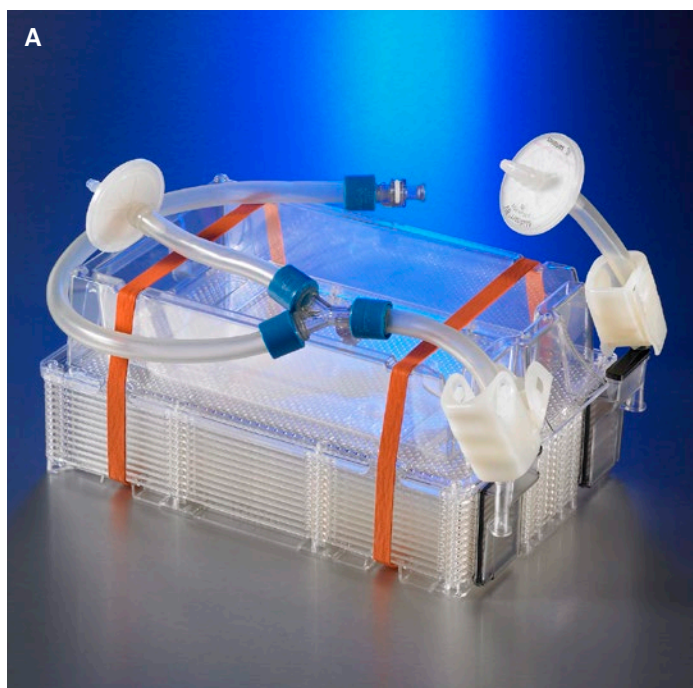


Figure 1. (A) Corning HYPERStack 12-layer cell culture vessel and (B) HYPERStack-36-layer cell culture vessel.

Materials and Methods

The following methods all included Corning® HYPERFlask® cell culture vessels and Corning CellBIND® surface CellSTACK® culture chambers as controls for the assessment of the Corning HYPERStack® vessels.

Cell Growth and Viability

Chinese Hamster Ovary (CHO-K1; ATCC Cat. No. CCL-61) and Madin Darby Bovine Kidney (MDBK; ATCC Cat. No. CRL-22) cells were seeded at 3,000 cells per cm², while Human Embryonic Kidney 293 (HEK-293; ATCC Cat. No. CRL-1573) cells were seeded at 5,000 cells per cm². The cell lines were cultured using Iscove's Modified Dulbecco's Medium (IMDM) (Corning Cat. No. 10-016-CMR), supplemented with 10% Fetal Bovine Serum (FBS) pre-warmed to room temperature. Cell suspensions were prepared and seeded in triplicate for each product type (Table 1) at a volume of 0.217 mL/cm² to achieve equivalent seeding densities for each vessel. Cultures were maintained in a humidified incubator set to 37°C and 5% CO₂. Daily samples of medium were taken and evaluated using the Nova BioProfile Flex® analyzer (Nova Biomedical) to monitor electrolytes, gas saturation, nutrient, and metabolite contents of the cultures. Cell morphology and overall growth was monitored visually using an Olympus inverted microscope. Due to the HYPERStack vessel's construction, a device was used to assist with the visualization of cells. Cells were harvested after incubation for 96 hours using room temperature trypsin/EDTA (Corning Cat. No. 25-052-CV) containing 0.1% Pluronic® F-68 (Corning Cat. No. 13-901-CI). To ensure that all cells were removed from the vessels, an additional phosphate buffered saline (PBS) wash was performed and collected. An additional vessel was set up for each study and stained with a crystal violet solution (Thermo Fisher Cat. No. 23-750025) to assess equal cell distribution within individual layers. Studies were repeated three times independently.

CO₂-independent Growth

Vero cells (ATCC Cat. No. CRL-1586) were adapted to CO₂-independent conditions by growing them in Leibovitz L-15 medium (L-15), (Lonza Cat. No. 12-700Q) supplemented with 4 mmol L-glutamine (Corning Cat. No. 25-005-CI) and 10% FBS.

Cells were seeded into each vessel at 3,000 cells per cm² in 0.217 mL/cm² (Table 1) using the controls stated above. To maintain a CO₂-independent system during culture, CellSTACK culture chambers were fitted with solid caps. Cultures were maintained for 96 hours in a warm room at 37°C and 20% relative humidity (RH). Daily samples of medium were acquired and evaluated using the Nova BioProfile Flex analyzer. Cultures were monitored and harvested as described above for cell growth testing.

Functional Testing

Early passage HEK-293 cells were seeded into a HYPERStack 12-layer vessel or 2-layer CellSTACK culture chamber following the method described above. Upon reaching 90% confluence, vessels were harvested and total cell yields determined. Cell counts and viability were obtained through the trypan blue exclusion method using the Nova BioProfile Flex analyzer. Following analysis, the cells were concentrated by centrifugation at 270 x g for 7 minutes. The cell pellets were resuspended in freezing media (10% DMSO + 90% growth media) to yield a final concentration of 5.0 x 10⁶ cells per vial and frozen down. To assess cellular function, cells from each test vessel were thawed into 3 mL of pre-warmed complete growth medium (10% FBS, IMDM). Cell concentration and viability were determined following centrifugation at 220 x g for 5 minutes to remove traces of DMSO. Cell pellets were resuspended in fresh growth media to a final concentration of 6.0 x 10⁵ cells/mL. A Fibronectin-coated 384-well Corning Epic® microplate (Corning Cat. No. 5042) was pre-warmed to room temperature, filled with 10 µL/well of growth medium then spun briefly in the centrifuge to remove trapped air. Half of the Epic microplate was filled with 30 µL/well of HYPERStack-12 cell suspension and the other half with 30 µL/well of CellSTACK cell suspension. After brief centrifugation, the microplate was incubated overnight in a humidified incubator at 37°C and 5% CO₂. The following day, the HEK-293 cellular response to SFLR (5 µM) (Bachem Cat. No. H-2938.0025), a Par-1 agonist, or Carbachol (50 µM) (Sigma Cat. No. C4382), an acetylcholine receptor agonist, was evaluated using the label-free Epic assay. The Epic technology is a label-free noninvasive biosensor system that is centered around resonant waveguide grating biosensors.

Table 1. Scale-up Product Information

Corning Cat. No.	Description	Purpose	Surface Area (cm ²)	Seed Volume (mL)
3310	2-layer Corning CellSTACK chamber*	Standard control (for 12-layer vessel)	1,272	276
3320	10-layer Corning CellSTACK chamber**	Standard control (for 36-layer vessel)	6,360	1,380
10024	Corning HYPERFlask vessel	HYPER technology control	1,720	373 (+ 190 mL media to fill)
10012	Corning HYPERStack-12 vessel	Test vessel	6,000	1,300
10036	Corning HYPERStack-36 vessel	Test vessel	18,000	3,900

*Used as control when setting up HYPERStack-12 studies.

**Used as control when setting up HYPERStack-36 studies.

Results

Cell Growth and Viability

The capability of the Corning® HYPERStack® cell culture vessel to perform as a viable tool for closed system, large scale, adherent cell production was evaluated. Utilizing Corning's gas permeable technology enables compact vessel construction and provides a higher growth surface area (cm²) per cubic footprint of the vessel, ultimately providing for better use of valuable incubator space. To illustrate, a HYPERStack 12-layer vessel has a volumetric footprint similar to that of a 2-layer stacked cell culture vessel, but offers the growth surface area closer to that of a 10-layer stacked cell culture vessel (Table 2). Initial comparison of the HYPERStack vessel to control vessels was assessed through cell morphology, phenotype, distribution, and growth. Microscopic observation indicated no visible difference in morphology or distribution of cells and there were no phenotypic abnormalities observed (Figure 2). To evaluate the effect on cell morphology, density, and distribution of cells, the vessels were stained with crystal violet. Crystal violet is a histological dye that works by fixing cells to the growth surface and staining the cells purple. Crystal violet staining of confluent vessels revealed uniform cell distribution within the individual stacks and throughout the HYPERStack vessels (Figure 3).

Additional assessment was performed once cultures reached confluence and the cells were harvested. Trypan blue exclusion was used to determine cell density, viability, and cell counts were converted to cells/cm² to uniformly compare the three different vessels types. Based on cells/cm², the HYPERStack vessels performed equally to the control vessels, with respect to cell viability which was measured above 95% (Figures 4 and 5). In addition, there was typically approximately 3X higher total cell yields from the HYPERStack vessel when compared to control vessels of similar volumetric footprint (Figure 5).

Cell Growth Environment

To assess the performance of the HYPERStack vessel the growth environment was monitored daily. Media samples were taken from all vessels and analyzed for nutrient and metabolite concentrations, dissolved gases, and electrolyte values. For example, Figure 6 shows the results from CHO-K1 growth environments in the Corning HYPERStack 12-layer, Corning HYPERFlask®, and 2-layer Corning CellSTACK® vessels over a 96-hour time period. Figure 6A shows a typical initial electrolyte equilibration of the growth medium which is usually completed in 24 to 48 hours. Figure 6B shows the normal nutrient/metabolite environment observed over a 96-hour growth period. The results show nutrients depleted and

metabolites increased with cell proliferation. Figure 6C indicates the saturation of %CO₂ and %O₂ in the growth environment. Similar to the electrolytes, CO₂ equilibration of the media is complete after 24 to 48 hours. The HYPERStack vessel's growth environment mimics that of the HYPERFlask vessel, which is constructed using the same gas permeable technology. Once complete equilibration of the growth media occurs, both HYPER technology vessels behave comparably to the CellSTACK control, which uses the standard liquid gas interface within the vessel.

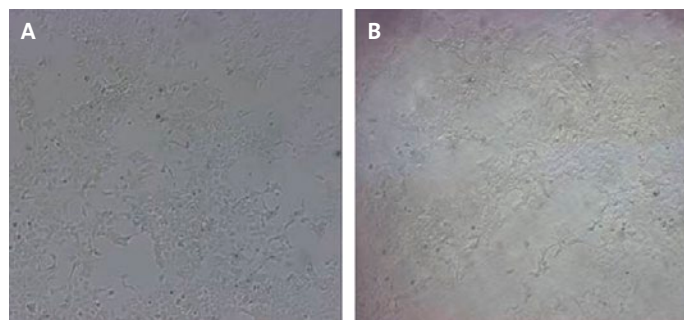


Figure 2. Visual analysis. Micrographs of HEK-293 cultures at 4X magnification using an inverted microscope. (A) 10-layer Corning CellSTACK and (B) Corning HYPERStack 12-layer vessel using a viewing device. Overall similar cell densities and morphology were observed.

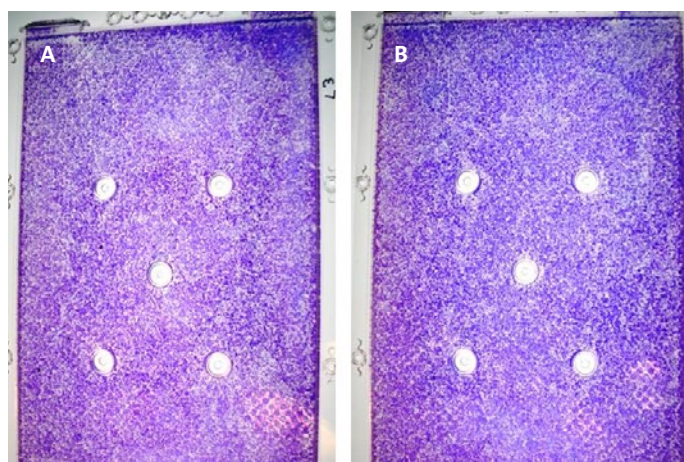


Figure 3. Cell distribution analysis. Crystal violet stained MBDK cultures after 96-hour incubation, third layer (A) and eighth layer (B) stacks from Corning HYPERStack 12-layer vessel. Staining demonstrated equal cell distribution throughout layers and within the vessel.

Table 2. Stacked Vessel Comparison

Footprint	Corning CellSTACK Chambers		Nunc® Cell Factory Chambers		Corning HYPERStack Vessels	
	No. Layers	Surface Area (cm ²)	No. Layers	Surface Area (cm ²)	No. Layers	Surface Area (cm ²)
2-stack	2	1,272	2	1,264	12	6,000
10-stack	10	6,360	10	6,320	36	18,000
40-stack	40	25,440	40	25,280	—	—

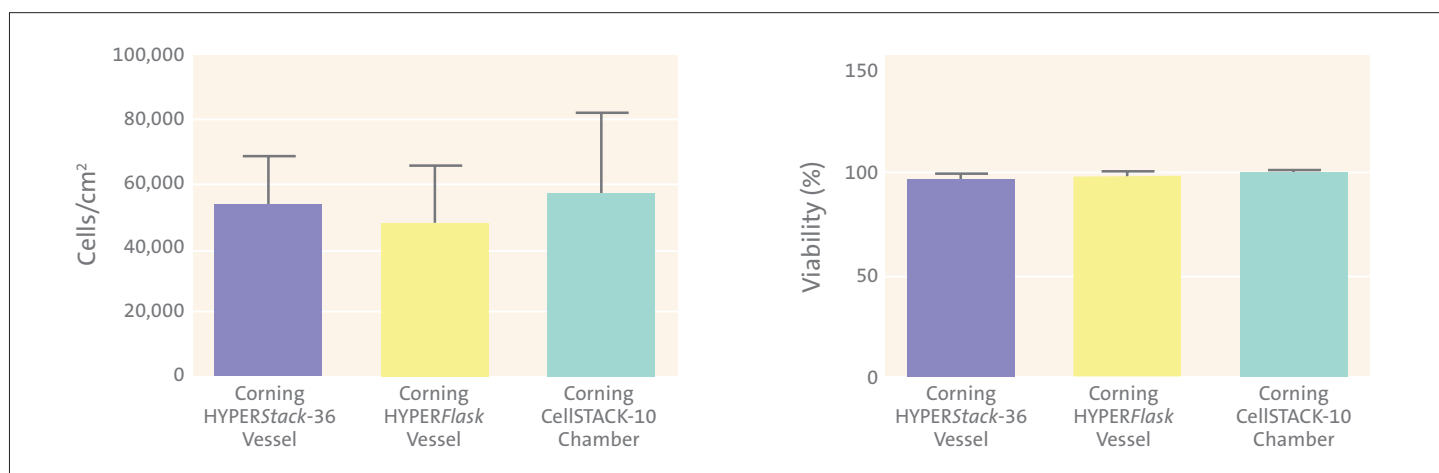


Figure 4. Cell growth analysis. HEK-293 cells cultured in Corning HYPERStack 36-layer, Corning CellSTACK 10-layer, and Corning HYPERFlask vessels. 96-hour incubation in a humidified incubator at 37°C and 5% CO₂. Paired T-test (0.166 and 0.678 values) indicated no statistical difference in cells/cm² between the vessels. Data encompassed nine vessels/condition from 3 independent studies.

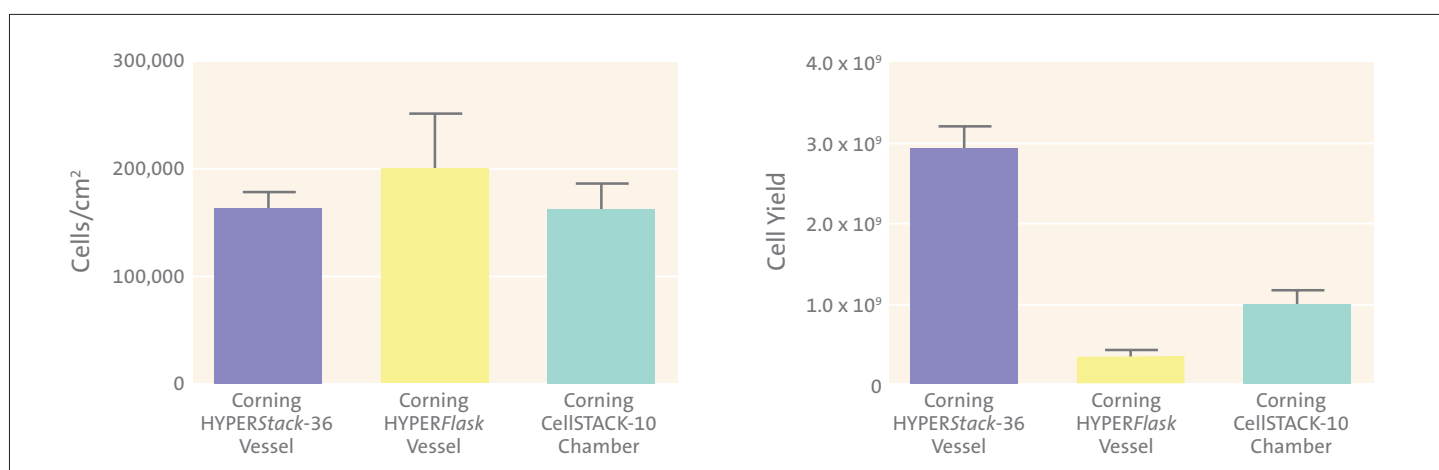


Figure 5. Cell growth analysis. CHO-K1 cells cultured in Corning HYPERStack 36-layer, Corning CellSTACK 10-layer, and Corning HYPERFlask vessels. 96-hour incubation at 37°C and 5% CO₂. Paired T-test (0.09 and 0.881 values) indicate no statistical difference in cell/cm² between the vessels. Total cell HYPERStack-36 yields are 3X greater than the 10-layer CellSTACK chamber. Encompassed data are from 3 independent studies.

CO₂-independent Growth

For many large-scale bioprocess environments, vessels used to culture cells are incubated in warm rooms under atmospheric conditions, due to the size and scale of the cultures. The ability to use the Corning® HYPERStack® vessel in this environment can increase the value to the end-user by increasing cell production and eliminating the need for additional incubator space. Because Corning's HYPER technology uses gas permeable film, it is necessary to use CO₂-independent media, formulated to buffer without the use of sodium bicarbonate and compatible with atmospheric conditions. To evaluate the performance of the HYPERStack vessel in a warm room environment, HYPERStack and control vessels were seeded with Vero cells adapted to grow in L-15 medium. L-15 medium is specifically formulated to sustain cell growth in an atmospheric CO₂ environment by buffering the environment

with phosphates, free base amino acids, galactose, and sodium pyruvate. Based on cells/cm² recovered per vessel and analysis of this data using the paired T-test, there was no significant difference (p-test values; 0.643 to Corning HYPERFlask® and 0.660 to Corning CellSTACK®) in the rate of cell growth between the three cell culture vessels (Figure 7A). Total HYPERStack vessel yields were 4X higher than stacked control vessel of similar volumetric footprint (Figure 7B).

Functional Testing

Due to the unique environment found in the HYPER technology, where cells grow directly on the gas permeable film, it is important to understand any changes in cell behavior and physiology. Corning Epic® label-free technology, a highly sensitive method for detecting changes in cellular response using an optical biosensor, was employed to evaluate functional changes in cells cultured

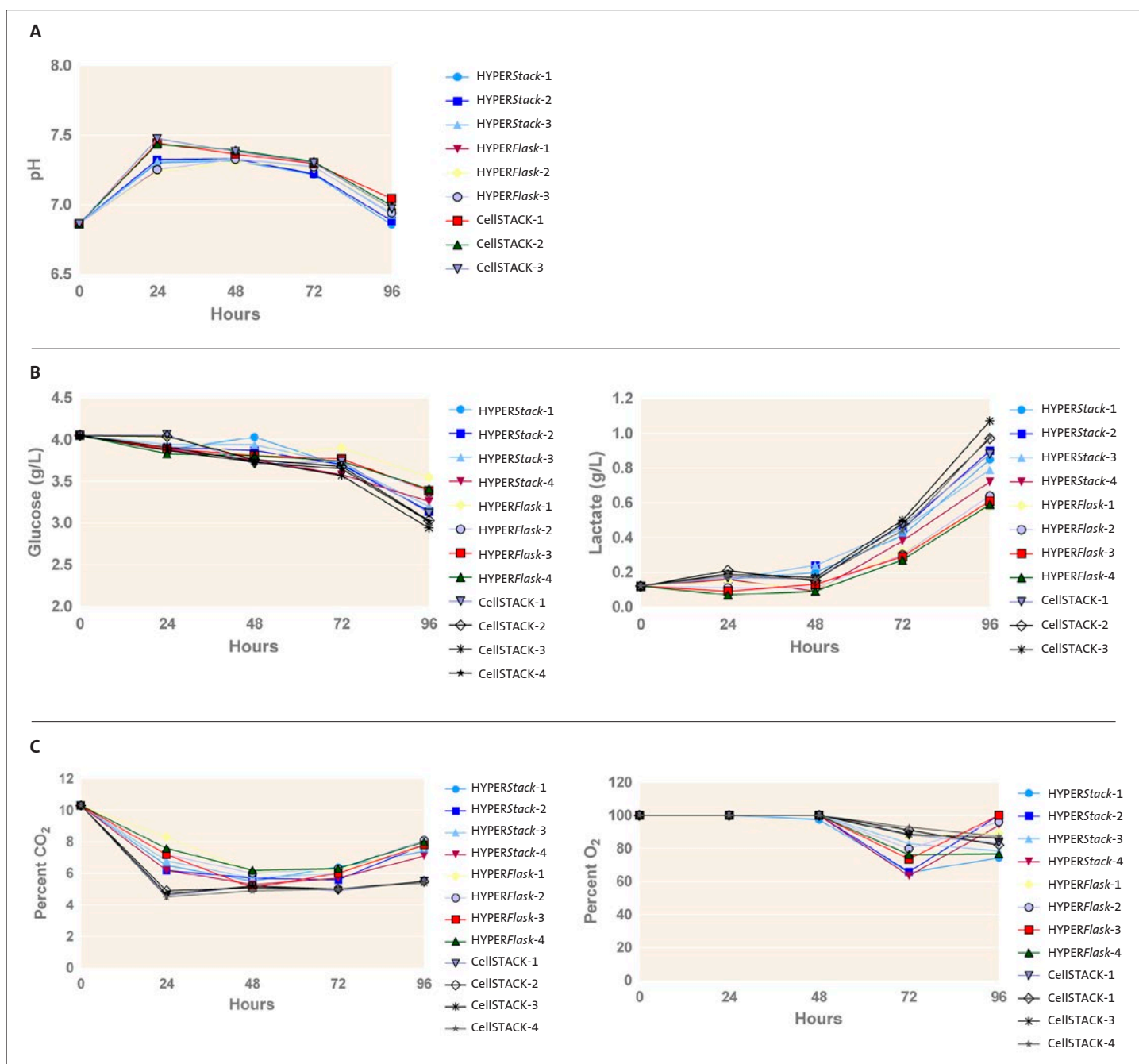


Figure 6. Analysis of the cell growth environment. Representative graphs of CHO-K1 cultures over a 96-hour period. (A) Electrolyte monitoring. pH from all three cultures equilibrated similarly after 48 hours and maintained for the duration of the study. (B) Nutrient and metabolite monitoring. Similar depletion of glucose (g/L) and buildup of lactate (g/L) by all three products. (C) Gas monitoring. Similar equilibration of CO₂ after 24-hour incubation and saturation of %O₂.

from two different vessel environments. Using the Corning® Epic® label-free technology, changes in HEK-293 cell physiology were evaluated after challenge with either SFLLR (5 μ M), a Par-1 agonist and carbachol (50 μ M), an acetylcholine receptor agonist. After the addition of the test compound, the responses to these stimuli were compared after initial growth in either Corning HYPERStack® vessels or Corning CellSTACK® vessels. HEK-293 cell were cryopreserved, thawed, and immediately used

in a cellular response assay. The results indicate no statistically significant changes in cellular function in response to SFLLR or carbachol challenge between cells derived from the HYPERStack or CellSTACK vessels (Figures 8A and 8B). Additionally, the data demonstrate no difference in two important metrics of assay reliability; %CV values and Z' values (Figure 8C). If either vessel impacted overall cellular health and physiology, both metrics could be indirectly altered.

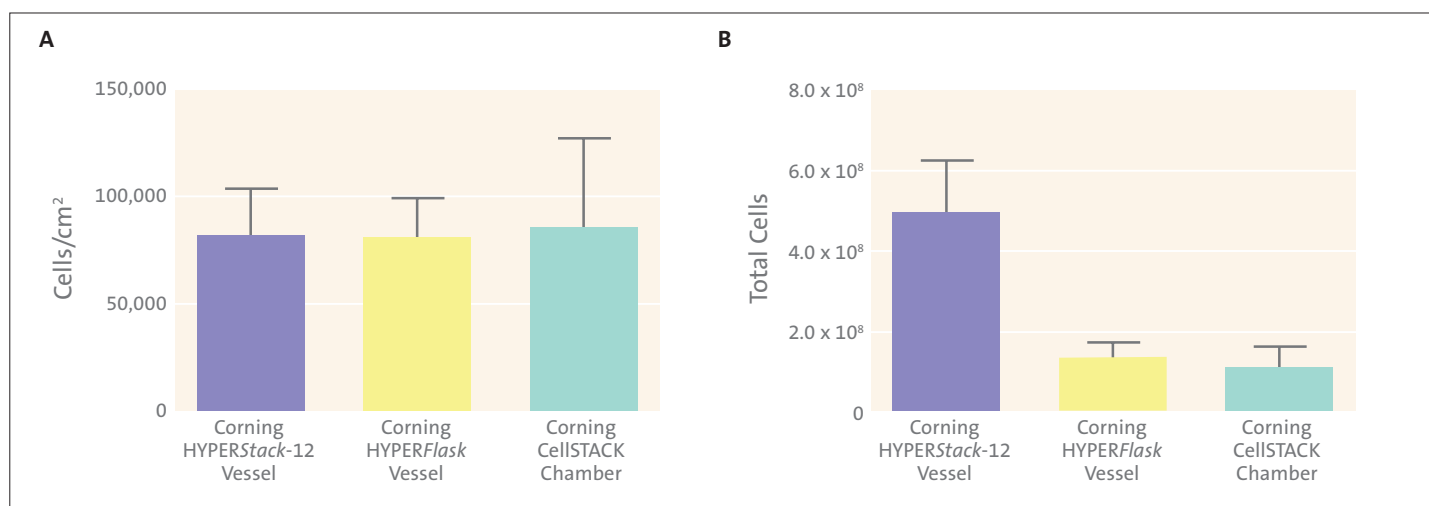


Figure 7. CO₂-independent growth analysis. (A) Vero cultures incubated in warm room under atmospheric conditions for 96 hours. Paired T-test indicated no significant difference in cells/cm² between the three products tested. Data shown is the average of three independent experiments (n = 9). Error bars represent ± S.D. (B) Total Corning® HYPERStack® vessel yields were 4X greater than yields from Corning CellSTACK® vessels with similar cubic footprint.

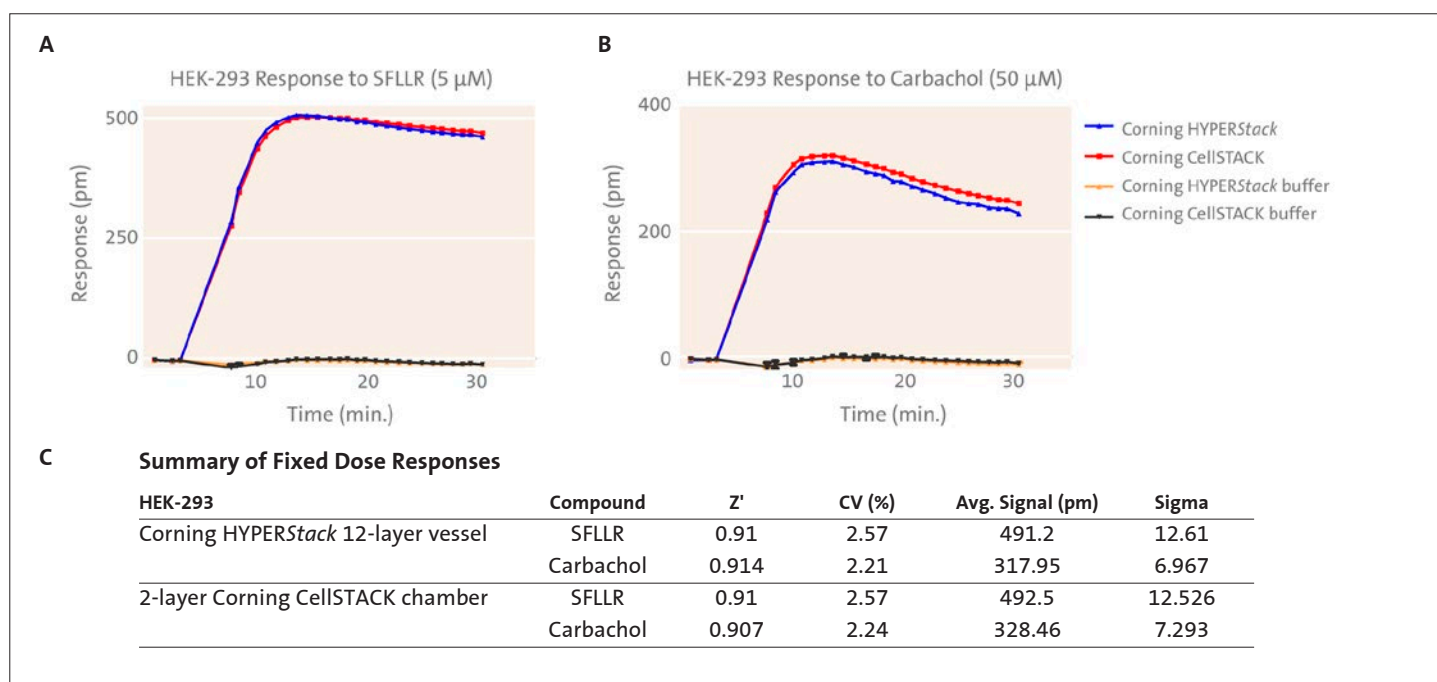


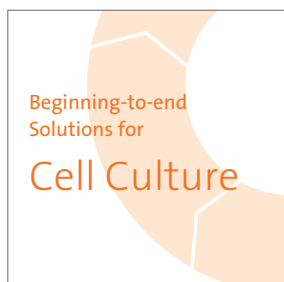
Figure 8. Functional analysis. Corning Epic® label-free analysis of HEK-293 cultures derived from Corning HYPERStack and Corning CellSTACK vessels. (A) No detectable difference in cellular response SFLLR (5 µM) between the two conditions. (B) No difference in cellular response to carbachol (50 µM) between the two conditions. (C) Summary data of Epic label-free analysis.

Summary of Analysis

- ▶ Based on analysis of growth environment, visual inspection of cell morphology and cells/cm² yield, the Corning® *HYPERStack*® vessel performance was equal to that of the Corning *HYPERFlask*® and Corning *CellSTACK*® control vessels.
- ▶ Based on total cell yield, the *HYPERStack* vessel produced 2.5X to 3X higher cell numbers than control vessels with similar spatial footprints.
- ▶ When used with the CO₂-independent medium, *HYPERStack* vessels can be successfully used in warm room environment for production of large numbers of adherent cells or cell products.
- ▶ Based on cellular response obtained by using the Corning *Epic*® label-free system, cells derived from a *HYPERStack* vessel performed comparably to those derived from a *CellSTACK* culture chamber.

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