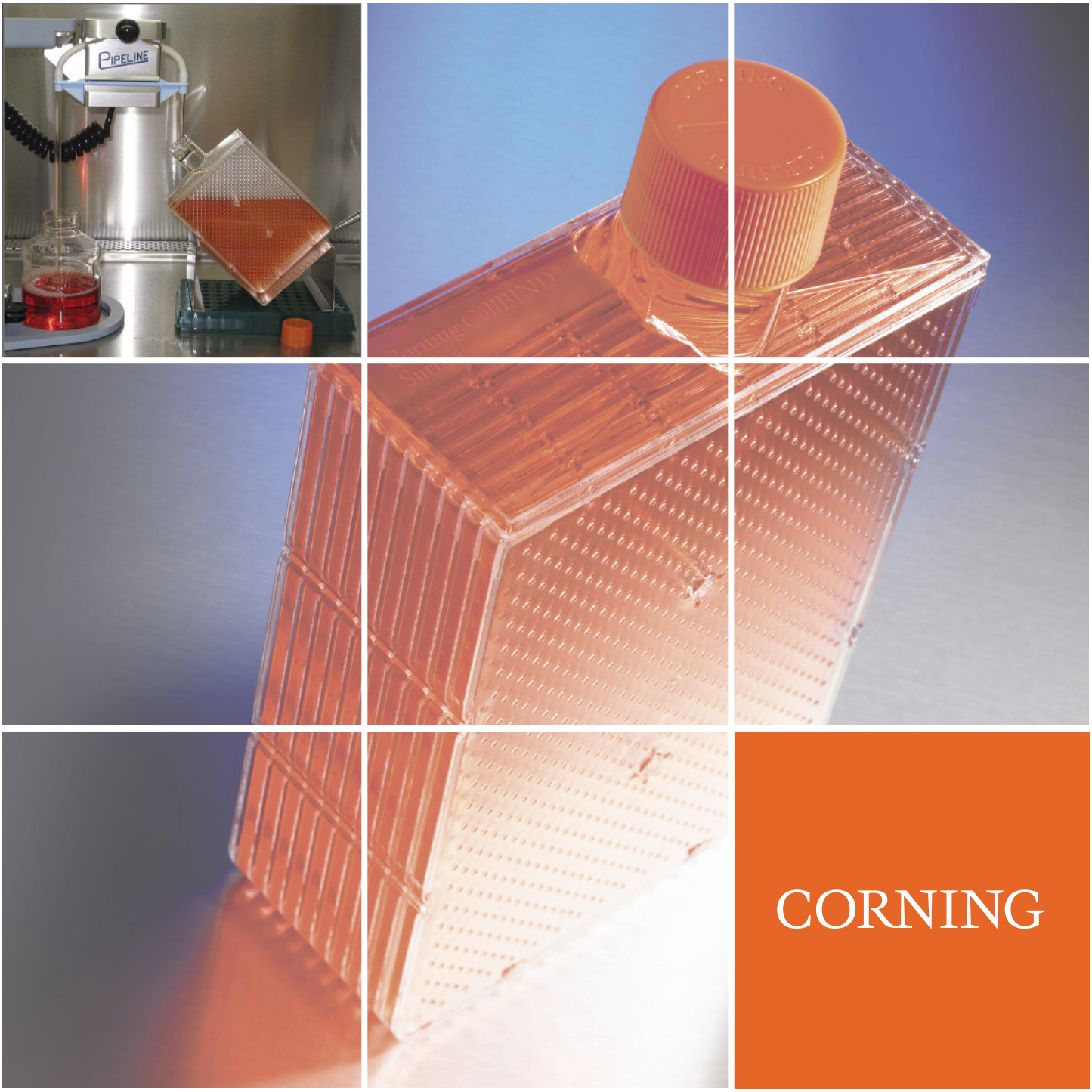
# Automated Cell Dispensing into Corning® HYPER*Flask*® Cell Culture Vessels Using Essen Instruments Pipeline Dispenser™

Protocol



## Introduction

The Corning® **H**igh **Y**ield **PER**formance Flask (HYPER*Flask*®) cell culture vessel (Figure 1) was developed to increase the number and quality of cells required by scientists in various types of laboratories, including High Throughput Screening. The multilayered HYPER*Flask* vessel uses a gas permeable film to provide gas exchange between the internal culture environment and the external atmospheric environment. The unique film design gives a 1720 $cm^2$ surface area, which is approximately 10 times that of a normal T-175 flask. The HYPER*Flask* vessel can be completely filled with medium and capped with a plug sealed cap that does not require venting. To learn more about how to use the HYPER*Flask* vessel, visit Corning's website **www.corning.com/lifesciences** to view literature and a user video.

Scientists interested in automating the HYPER*Flask* vessel process have limited options in terms of technology. This protocol will describe one automation method using the Pipeline Dispenser from Essen Instruments, Inc. (Ann Arbor, MI). The Pipeline Dispenser provides a fast, accurate and efficient method to transfer medium into the HYPER*Flask* vessel (Figure 2), saving time and reducing fatigue.

## Protocol: Cell Seeding and Filling of the HYPER*Flask* Cell Culture Vessel

The HYPER*Flask* vessel is designed to be filled with medium, leaving no headspace (as normally found in a T-flask), with the final medium volume being 565 mL. For cell seeding densities, the recommendation for most cell types is 5.0 x $10^6$ to 1.72 x $10^7$ per flask or 0.3 to 1 x $10^4$ cells per $cm^2$. Your seeding density will vary depending on your cell type and culture duration requirements.

**Helpful Hint:** If choosing to pre-warm the vessel empty prior to seeding or when using low volumes during protocols such as trypsinization, transfections, etc. for prolonged periods of time (greater than 60 minutes), vent the vessel with the cap in the venting position as shown in Figure 3. This will allow proper ventilation to prevent pressure build-up. *Please note*: This includes storing the empty HYPER*Flask* vessels for periods of time greater than 60 minutes in the incubators of The Automation Partnership (TAP) SelecT™ and CompacT SelecT™ automated cell culture systems.

1. Prepare cell suspension at the desired cells/$cm^2$ in 565 mL growth medium. Make sure you set aside approximately 5 to 10 mL of media, without cells, to prime the Peripette.
2. Set up the Pipeline dispenser (we recommend testing the parameters detailed below with your culture medium prior to seeding a flask, the optimal volumes may differ depending on the unit).
   a. Carefully open the sterile Peripette in the culture hood and place in the Pipeline dispenser as described by the manufacturer making sure that both ends remain sterile.
   b. Set the Pipeline dispenser parameters as shown in the instrument setting table. Press the Menu button on the front panel to step through each mode and the **Enter** button to accept each entry.

**Instrument Settings**

| | | |
|---|---|---|
| i. | Dispense Mode: | Auto |
| ii. | Using the arrows, enter the dispensing volume: (the remaining 30 mL will be dispensed manually) | 535 mL |
| iii. | Dispensing Speed: | Slow |

   c. Once you have finished setting up the dispenser the alpha display will read **–SET–** to indicate a successful update of the parameters.

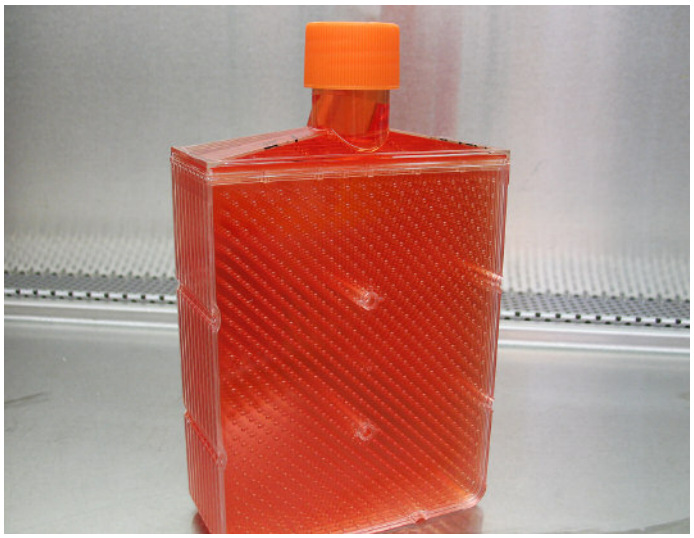


**Figure 1.** HYPER*Flask* vessel filled with 565 mL medium.


**Figure 2.** The proper placement and setup of the HYPER*Flask* vessel and media bottle in the Pipeline™ Dispenser from Essen Instruments, Inc.

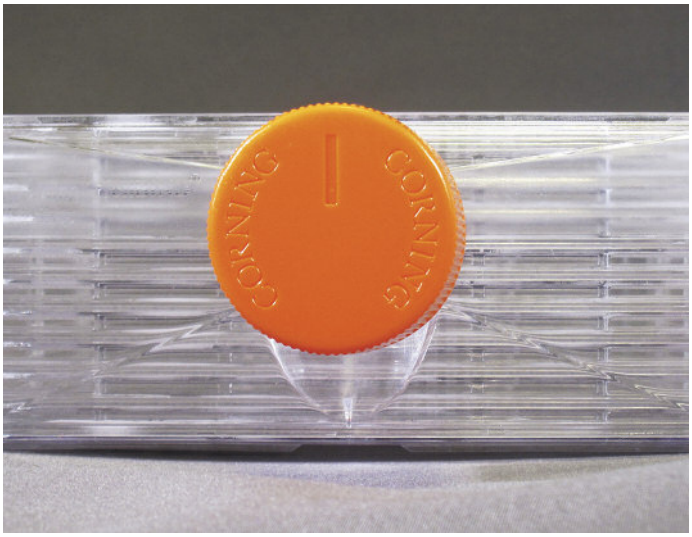



**Figure 3.** Venting position to be used when pre-warming the HYPER*Flask*® and HYPER*Flask* M vessels when the vessel is empty or contains low volumes of liquid for prolonged periods of time (greater than 60 minutes).

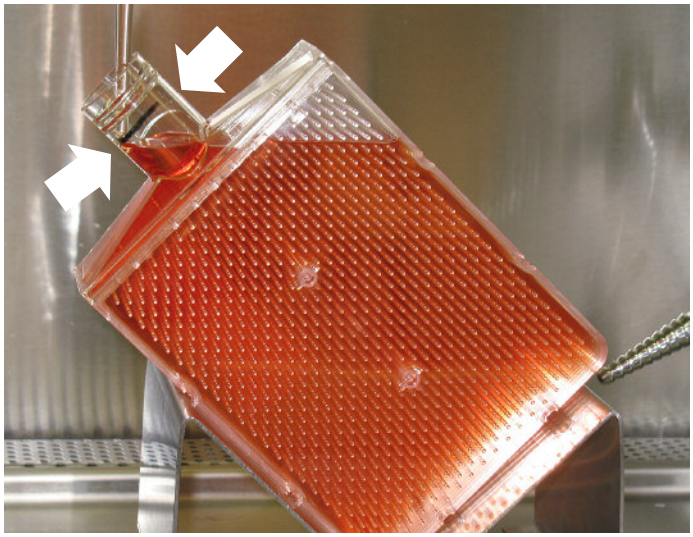


**Figure 4.** Arrows show the placement of the dispensing end of the Peripette in the HYPER*Flask* vessel neck.

3. Using the manufacturer's directions, prime the Peripette with culture medium without any cells.
4. Place the cell suspension in the media place holder and the HYPER*Flask* vessel, tilted at a 45° angle as shown in Figure 2. The HYPER*Flask* vessel should be placed at a height approximately 3.5 cm from the base of the cell culture hood.
5. Lower the Peripette so that the filling end is at or near the bottom of the cell suspension container and the dispensing end inside the HYPER*Flask* vessel neck so that it touches the inside portion of the neck. (Refer to the black line and arrows in Figure 4.)
6. Press the **START** button. At the slow setting it should take approximately 4 to 5 minutes to complete dispensing the 535 mL of cell suspension.
7. Lift the Peripette up and place the HYPER*Flask* vessel in an upright position. Although there should very little air bubbles present, gently tap the flask to dislodge any that may be in between layers. We recommend that you add the remaining 30 mL of cell suspension to bring the fluid level equal to the lowest thread on the neck (Figure 5). If excessive bubbles are present, aspirate or pipet out the bubbles and add more medium to bring the volume to the lowest cap thread.
8. Cap the flask tightly; making sure it is tightened past the detent feature and place in the incubator. For more detailed instructions on using the HYPER*Flask* vessel refer to **www.corning.com/lifesciences**.

   **Helpful Hint:** Due to the direct contact of the vessel cap with culture medium, it is recommended to change the cap when culturing for prolonged periods of time or when opening and closing the vessel repeatedly. This will help to reduce the possibility of contamination and ensure that a sufficient seal is obtained. For your convenience, additional caps are available (Cat. No. 10035).

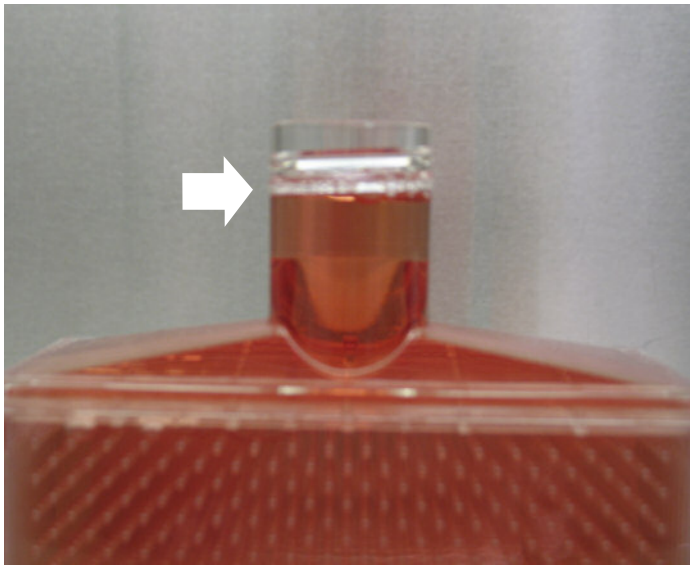


**Figure 5.** Arrow shows the proper medium level (at the first cap thread) prior to adding the cap.

This protocol was validated using two different cell lines, HepG2 (ATCC® No. HB-8065™) and 59 M alpha3-18 (ATCC No. CRL-10154™), both from ATCC, Manassas, VA. Both lines were seeded at a density of 10,000 cells/cm$^2$ and dispensed into HYPER*Flask* vessels using the Pipeline protocol described above. After 4 days, both culture lines were in excellent health with viabilities >95% (tested via trypan blue vital stain). We recommend dispensing the medium at slow speed to reduce the bubble formation and the potential splashing of the medium increasing the chance for contamination.

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