Corning® HYPER*Flask*® M Cell Culture Vessel

Instructions for Use

CORNING

Corning HYPER*Flask* M cell culture vessel

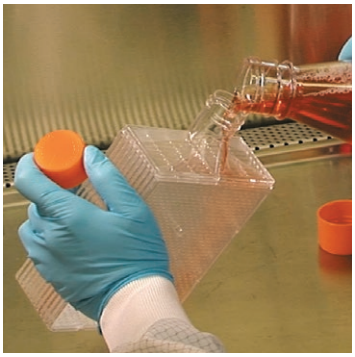


Figure 1. Pour carefully to avoid foaming and bubbles. Notice tilt of flask to achieve low foaming.

Introduction

The Corning **H**igh **Y**ield **PER**formance Flask **M**anual (HYPER*Flask* M) cell culture vessel is specifically designed for manual use. This is a multi-layer flask that uses a gas permeable film to provide gas exchange between the cells and culture medium and the atmospheric environment surrounding it. This allows for a much greater cell growth surface area within the traditional T-flask space. The HYPER*Flask* M vessel is designed to be filled entirely with medium and sealed with a solid cap. There is no need to crack the cap or use a vented cap when culturing the gas permeable film and air space between each of the 10 layers. The HYPER*Flask* M vessel has a total growth area of 1720 cm^2 or 10 times the growth area of a standard T-175 flask. The protocol below is a generalized protocol for routine or standard cell culture needs and will allow you to achieve the best results with the HYPER*Flask* M vessel and the original HYPER*Flask* vessel that is designed for use in automation. Both vessels have been successfully used for cell propagation (adherent and non-adherent), protein production, virus production, and transfection.

Initial Cell Seeding

The HYPER*Flask* M vessel is intended to be entirely filled with media leaving no headspace as typically found in T-flasks. Accordingly, the final fill volume of the HYPER*Flask* M vessel is 560 to 565 mL of media. An initial seeding inoculum of 5.0 x 10^6 to 1.72 x 10^7 cells per flask (0.3 to 1 x 10^4 cells per cm^2) is recommended for most cell types. The seeding density will vary depending on the cell type, medium and culture duration needs. Begin with a seeding density, medium type and culture duration equivalent to that used in standard T-flasks or dishes for the cell type used.

1. Prepare the cell suspension at the desired cells/cm^2 in 500 mL growth medium.
2. Remove the cap and tilt the flask on one of the two bottom edges to about 60°. Carefully and slowly pour the entire cell suspension down the side of the manifold favoring the canted neck and avoiding the air dam on the top side of the neck (Figure 1). While pouring, try to make as much contact with the inside of the neck as possible. The greater the amount of surface area of the HYPER*Flask* M vessel neck is contacted the less bubble formation will occur. The adapter grid of the HYPER*Flask* M vessel allows for fluid and air to travel separate flow paths. This decreases foam generation when filling or emptying when using the angled technique previously described. If foam generation does occur, stop pouring, and aspirate bubbles. Readjust your pouring angle until no bubbles are made.
   - The HYPER*Flask* M vessel can also be filled and emptied by pipetting.
   - If choosing to pre-warm the empty vessel prior to seeding, or when incubating with a low volume of Trypsin or other buffer for greater than 1 hour, it is recommended to vent the vessel in the venting position with a loosened cap as shown in Figure 2. This will allow proper ventilation to prevent pressure build-up. **NOTE:** This includes storing the empty HYPER*Flask* vessels for periods of time greater than 60 minutes in the incubators of Sartorius Stedim SelecT™ and CompacT SelecT™ automated cell culture systems.
3. As the vessel fills, slowly return the flask to an upright position to prevent media rushing out the neck from trapped air escaping.

Figure 2. Venting position to be used when pre-warming the Corning® 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).

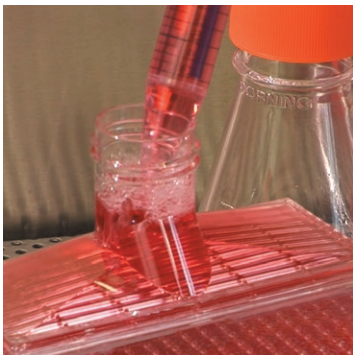


Figure 3. Add medium to the first cap thread before replacing cap.

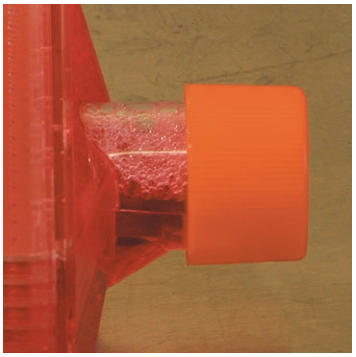


Figure 4. Placing the flask on end in the incubator and then lowering it slowly will keep any bubbles in the cap area.

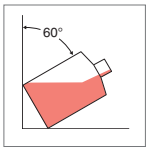
4. Tap the flask or gently bang on hard surface to dislodge any air bubbles that may be trapped in its layers.
5. Add medium (~60 mL) to bring the fluid level in the flask equal to the lowest thread on the neck (Figure 3). If excessive bubble formation occurs it may be necessary to aspirate or pipet out some of the bubbles and add medium to the lowest cap thread.
6. Cap the flask tightly by ensuring that the cap is tightened past the detent feature. Move to the horizontal position with the angled neck of the flask facing down (Figure 4). Confirm that all bubbles remain contained within the neck.

   NOTE: If some bubbles escape to top layer, stand flask upright, tap flask to migrate all bubbles into the neck and add medium to the top thread and repeat step 6. Small amounts of tiny bubbles in the top layer or manifold do not reduce or inhibit cell yields.

   - 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 (Corning Cat. No. 10035).

Alternative Method for Cell Seeding

1. Prepare a cell suspension with the number of cells necessary to achieve the desired seeding density for the entire flask in a small volume of growth medium. We recommend not going below a 50 mL seed volume.
2. Remove the cap and tilt the flask to about 60° (see diagram, right). Carefully and slowly pour the entire cell suspension down the angled side of the neck avoiding the air dam on the top side of the neck (Figure 1).



3. Close cap securely and lay the flask on its side to allow the solution to distribute evenly between layers. This ensures that each layer of the flask receives the same volume of liquid (Figure 5).
4. Return the flask to the upright position and continue filling as indicated in steps 2 through 6 in the **Initial Cell Seeding** section.

Cell Visualization

Cells grown in the HYPER*Flask* M vessel can be observed with a standard inverted microscope. Using a 4X objective (40X total magnification) it is possible to observe the two lowest layers of the flask. Additionally, the top two layers of the flask can be visualized when the flask is inverted. If using higher power objectives such as 10X and 20X, only the lowest (and top most) layer can be observed.

Medium Removal

The HYPER*Flask* M vessel has been specially designed for rapid and efficient fluid removal by pouring. Though this is the easiest and most rapid method, fluid can be removed by aspiration or pipetting.

1. Remove the cap and initially tilt the flask so that the medium is pouring over the air dam into a waste container (Figure 6). This means that the flask is initially being poured upside down with the angled portion of the neck and the bar code surface facing up. While pouring, slowly rotate the flask 180° until the medium is flowing down the angled neck of the flask. Carefully adjust the pouring angle to avoid excessive bubbling and foaming.
2. Gently rock the flask back and forth to eliminate any remaining liquid utilizing the liquid guide located at the base of the neck.

   - If any droplets of medium remain around the neck opening, they can be easily removed with a sterile gauze pad or alcohol wipe.

   NOTE: For loosely adherent cells such as HEK-293 cells, care must be taken to ensure that cells remain attached. If cells come off in the waste media or PBS wash, collection may be necessary.

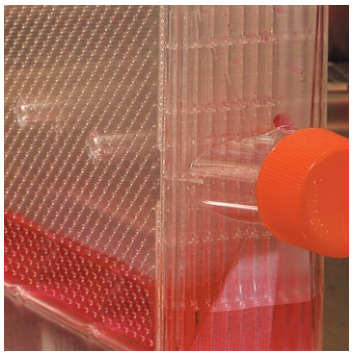


Figure 5. Any liquid inside the flask will distribute evenly into each layer of the flask when it is placed on its side.

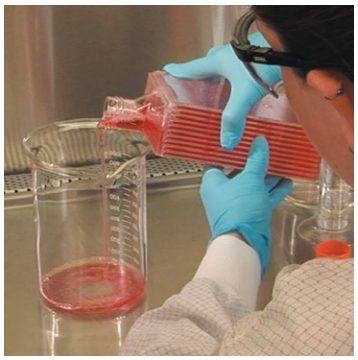


Figure 6. This initial pouring position allows medium to flow over the air dam and reduces foaming.

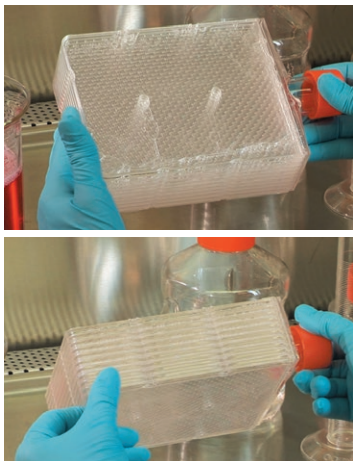


Figure 7. Rotating the flask several times back and forth will help cells to come in contact with the rinsing or dissociation solution completely.

Harvesting Cells

Harvesting of cells from Corning® HYPER*Flask*® M vessels is very similar to standard procedures for T-flasks with a few special handling considerations, which are detailed in the following steps and notes. To remove most cells from the surface, we strongly recommend solutions such as Accutase® and HyQtase®, or other collagenase based reagents. These reagents are less stressful on the cells and allow extended incubations while maintaining cell viability and cell surface receptors. The steps outlined below are generalized steps that are suitable for most cell types, i.e., CHO, HeLa, Vero, etc. For cells that are either loosely adherent or strongly adherent some special handling is required and is outlined below.

- If trypsin is used, closely monitor cell release as for most cell types the time for dissociation is 25% to 50% less than standard T-flasks. Decreasing incubation will prevent cell lysis and clumping from over-trypsinization.
- For loosely adherent cells such as HEK-293 cells, tapping the flask during the rinse step is generally sufficient to remove the cells. There is no subsequent need for dissociation reagents unless cells remain attached.
- For strongly adherent cells such as MDCK cells, incubation of the flask with PBS during the rinse step for 10 to 15 minutes can decrease dissociation time and help prevent over-trypsinization. The PBS can then be removed, and steps 5 through 12 can be followed.

1. Remove culture medium as described above.
2. Add 100 mL rinsing solution (PBS).
3. Cap the flask and lay it on its side to allow the solution to distribute evenly between layers. This ensures that each layer of the flask receives the same volume of liquid (Figure 5).
4. Gently rotate the flask back and forth 180° along its long axis several times so that the entire cell sheet in each layer is thoroughly rinsed (Figure 7).

   NOTE: Although it is difficult to observe, 4 or more rotations are sufficient to completely cover/rinse all the growth surfaces.

   - To increase the effectiveness of coating all surfaces it is possible to equilibrate the liquid to a subset of layers before rotating the flask to cover the layers. To do this, start with the flask in the incubation position and tilt the bottom of the flask up and towards the cap. The liquid should pool to the corner of the manifold and be in the bottom few layers. Gently rotate flask as in step 4. Turn the flask over and repeat pooling and rocking as before to wash the top layers.
5. Pour out the rinsing solution and replace with 50 to 100 mL of dissociation solution.
   - If desired, 50 mL of dissociation solution can be combined with 50 mL of diluent (such as PBS) to increase volume. This has been successfully done with trypsin, HyQtase, and Accutase.
6. Repeat **Harvest** steps 3 and 4, to evenly expose the cells to the dissociation solution. If desired, lay the flask in an incubator to facilitate cell detachment.
   - **Do not** incubate flask for longer than 60 minutes under these conditions. For incubations longer than 60 minutes, pre-equilibration of reagents to room temperature or warmer is required.
   - The formation of bubbles during this and subsequent harvest steps is normal. The bubbles do not reduce or impact harvest efficiency.
7. Once most of the cells become rounded and/or dislodged, shake or tap the flask sharply to dislodge the remaining cells. Cell detachment can be visually monitored by using a microscope.
   - To aid removal of rounded cells as well as to reduce bubbles, equilibrate the dissociation solution to all layers and in a swift motion swing flask downward forcing the liquid to the other side of flask and across the layers. Repeat as necessary.

8. Add inactivating solution to the Corning® HYPER*Flask*® M vessel, equilibrate, rinse and collect into a suitable collection vessel, such as a 250 mL centrifuge tube (Corning Cat. No. 430776) or storage bottle (Corning Cat. No. 430281).
   - To reduce foaming, a collection vessel can be set up ahead of time with an equal volume of inactivating solution such as serum containing medium. The cell suspension can be poured directly into this container to avoid reintroducing serum into the HYPER*Flask* M vessel.
9. If preferred, cell recovery can be improved slightly by performing a wash by adding 100 mL of rinsing solution, such as PBS, to the flask. For most cell types, this represents ~1% to 2% of the total cell harvest.
10. Repeat Harvest steps 3 and 4.
11. Pour into same collection vessel as step 8. It may be necessary to pipet the cell suspension up and down (trituration) to break up any cell clumps.

For more specific information on claims, visit the Certificates page at **www.corning.com/lifesciences**.

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