Corning® HYPER*Flask*™ Cell Culture Vessel

Instructions for Use

Corning HYPER*Flask* Cell Culture Vessel

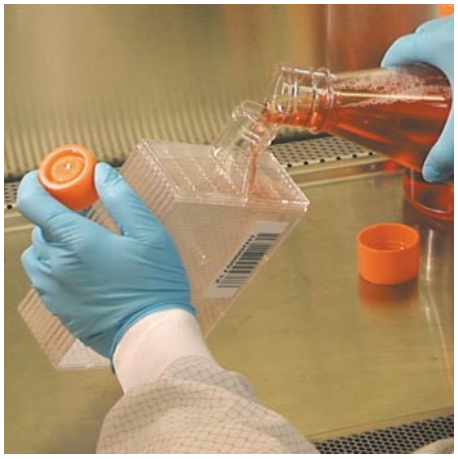

Figure 1. Pour carefully to avoid foaming and bubbles. Notice tilt of flask to achieve low foaming.

Introduction

The Corning HYPER*Flask* vessel is a new multilayer 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 as in the traditional T-flask space. The HYPER*Flask* 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 due to the nature of the gas permeable film. The HYPER*Flask* vessel has a total growth area of 1720 cm^2 or 10 times the growth area of a standard T175 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* vessel. It has been successfully used for cell propagation (adherent and non-adherent), protein production, virus production and transfections. As an aid to these instructions, visit **www.corning.com/lifesciences** to view the Corning HYPER*Flask* Vessel Video Guide.

Initial Cell Seeding

The HYPER*Flask* 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* vessel is 560 to 565 mL of media. An initial seeding inoculum of 5.0×10^6 to 1.72×10^7 cells per flask (0.3 to 1×10^4 cells per cm^2) is recommended for most cell types. Your seeding density will vary depending on your cell type, medium used and culture duration needs. Begin with a seeding density, medium type and culture duration equivalent to that used in standard T-flasks or dishes in your lab 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 60º. 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). The greater surface area of the HYPER*Flask* neck utilized to pour medium down, the less buble formation will occur.

   HELPFUL HINT: The HYPER*Flask* vessel can also be filled and emptied by pipetting.
3. As the vessel fills, slowly return the flask to an upright position to avoid trapped air.
4. Gently tap the flask to dislodge any air bubbles that may be trapped in its layers.

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Figure 2. Add medium to the first cap thread before replacing cap.

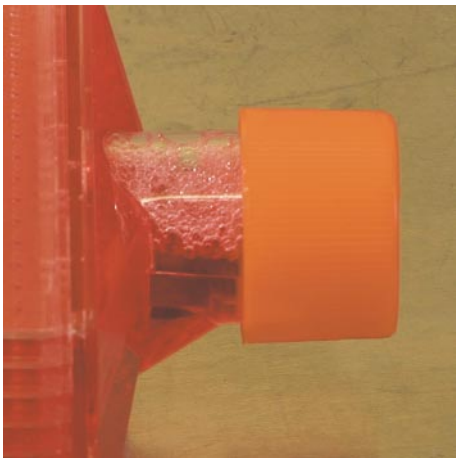

Figure 3. Placing the flask on end in the incubator and then lowering it slowly will keep any bubbles in the cap area.

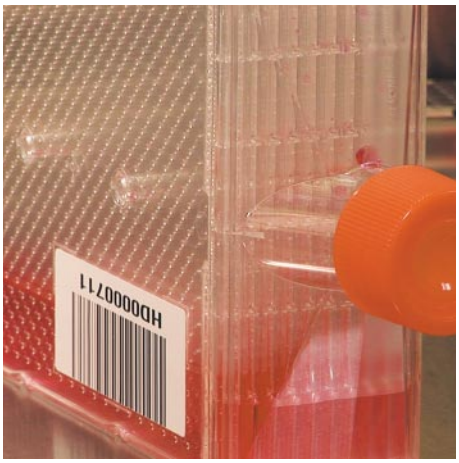

Figure 4. Any liquid inside the flask will distribute evenly into each layer of the flask when it is placed on its side.

5. Add medium (~60 mL) to bring the fluid level in the flask equal to the lowest thread on the neck (Figure 2). 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 3). 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.

Alternative Method for Cell Seeding

1. Prepare a cell suspension with the amount 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 60º. 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. Tightly 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 4).
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*™ 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* vessel has been specially designed for rapid and efficient fluid removal by pouring. Though this is the most rapid and easiest method, fluid can be removed by aspiration or pipeting.

1. Remove the cap and initially tilt the flask so that the medium is pouring over the air dam into a waste container (Figure 5). 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.
2. 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.
3. Gently rock the flask back and forth to eliminate any remaining liquid.

   HELPFUL HINT: If any droplets of medium remain around the neck opening they can be easily removed with a sterile gauze pad.

   NOTE: For loosely adherent cells such as HEK293 cells, care must be taken to ensure that cells remain attached.

Harvesting Cells

Harvesting of cells from HYPER*Flask*™ vessels is very similar to standard procedures for T-flasks with a few special handling considerations, which are detailed in the steps and notes below. 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 in the notes below.

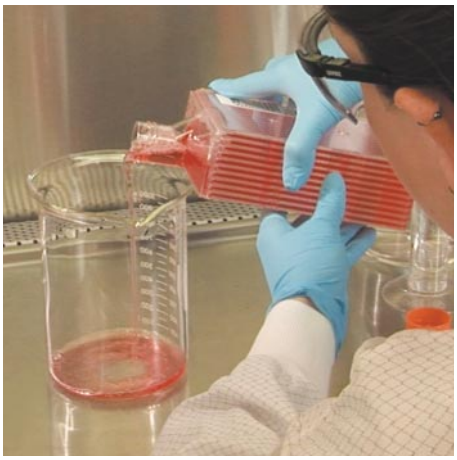

Figure 5. This initial pouring position allows medium to flow over the air dam and reduces foaming.

HELPFUL HINT: If trypsin is used, closely monitor cell release as for most cell types the time for dissociation is 25 to 50% reduced relative to standard T-flasks. This will prevent cell lysis and clumping from over-trypsinization.

NOTE 1: For loosely adherent cells such as HEK293 cells, tapping the flask during the rinse step is generally sufficient to remove the cells. There is no subsequent need for dissociating reagents.

NOTE 2: For strongly adherent cells such as MDCK cells, incubation of the flask with PBS/EDTA 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/EDTA).
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 4).
4. Gently rotate the flask back and forth 180º along its long axis several times so that the entire cell sheet in each layer is thoroughly rinsed (Figure 6).

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

   HELPFUL HINT: 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 you. The liquid should pool to the corner of the manifold. Gently rotate flask as in step 4. Turn the flask over and repeat pooling and rocking as before.
5. Pour out the rinsing solution and replace with 50 to 100 mL of dissociation solution.

   HELPFUL HINT: If desired, 50 mL of dissociation solution can be combined with 50 mL of diluent (such as PBS/EDTA) 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.

   NOTE 1: 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.

   NOTE 2: The formation of bubbles during this and subsequent harvest steps is normal. The bubbles do not reduce or impact harvest efficiency.

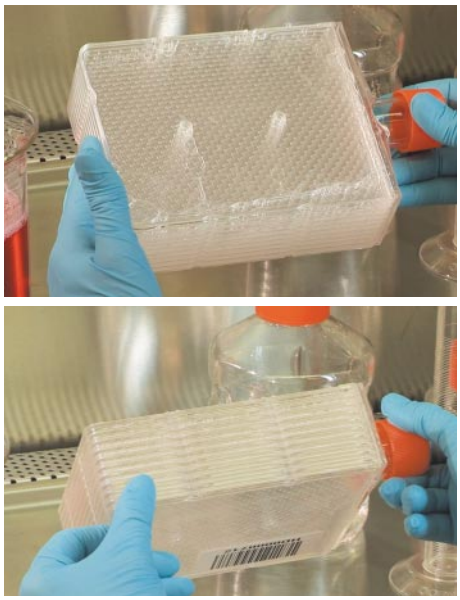

Figure 6. Rotating the flask several times back and forth will completely expose all of the cells to the rinsing or dissociating solution.

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.

   HELPFUL HINT: 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. Pour off cell suspension to a suitable collection vessel, such as a 250 mL centrifuge (Cat. No. 430776) or storage bottle (Cat. No. 430281). If using trypsin, the collection vessel should contain an equal volume of inactivating solution such as serum containing medium.
9. If preferred, cell recovery can be improved slightly by performing a wash by adding 100 mL of rinsing solution, such as PBS/EDTA, 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.

Please visit the Corning Life Sciences web site to view a video presentation that describes the proper handling of the HYPER*Flask*™ Cell Culture Vessel.

For additional product or technical information, please e-mail us at CLStechserv@corning.com, visit our web site **www.corning.com/lifesciences**, or call 1.800.492.1110. Outside the United States, call 978.442.2200.

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