Corning® HYPER*Flask*® Cell Culture Vessels

Low Volume Harvest Protocol

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Introduction

With a growth area of 1720 cm^2, Corning's new family of **H**igh **Y**ield **Per**formance Flasks (HYPER*Flask* and HYPER*Flask* M cell culture vessels) are ideal for the quick scale-up of large numbers of cells. Currently there are a number of applications such as electroporation, viral infection, and several HTS assays that require concentrated cell suspensions (> $4e^6$ cells/mL). To this end, we have developed a harvesting protocol for the HYPER*Flask* cell culture vessels that will enable users to achieve a concentrated cell suspension suitable for use in such applications.

Initial Cell Seeding

The HYPER*Flask* cell culture vessels are innovative new flasks that require slight modifications in handling when compared to a standard T-flask. For optimal use and handling of the HYPER*Flask* vessel, please refer to the HYPER*Flask* Cell Culture Vessel Instructions for Use manual. The HYPER*Flask* vessel requires approximately 560 mL of medium to fill completely. Cell seeding density will vary depending on cell type, but in general, an initial seeding inoculum of 5.0×10^6 to 1.72×10^7 cells/flask (0.3 to 1×10^4 cells/cm^2) is recommended. For an even cell distribution, prepare cell suspension in 500 mL of medium volume, mix well and carefully pour into the flask. Bring final volume in the vessel to 560 mL using fresh growth media (refer to Figures 1-5 of Instructions for Use manual).

Removing Solutions

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 also be removed by aspiration or pipeting.

Pouring Technique *(refer to Figure 5 of Instructions for Use manual)*

1. Remove the cap and initially tilt the vessel (bar code up) so that the medium is pouring over the air dam into a waste container.
2. While pouring, slowly rotate the vessel 180° until the medium is flowing down the angled neck. Carefully adjust the pouring angle to avoid excessive bubbling and foaming.
3. Gently rock the vessel back and forth to eliminate any remaining liquid. Once the medium is fully drained, any droplets of medium remaining around the neck opening can be removed with a sterile gauze pad.

Aspirating Technique

1. Remove cap and place aspirating pipette inside neck, angled down where the body meets the canted neck.
2. Gently tilt flask to guide liquid towards tip of pipette as liquid level decreases until all liquid has been aspirated out of the flask. Exercise caution not to tilt the flask excessively to avoid spilling the medium.
3. Gently rock the flask back and forth to eliminate any remaining liquid.

Low Volume Harvest

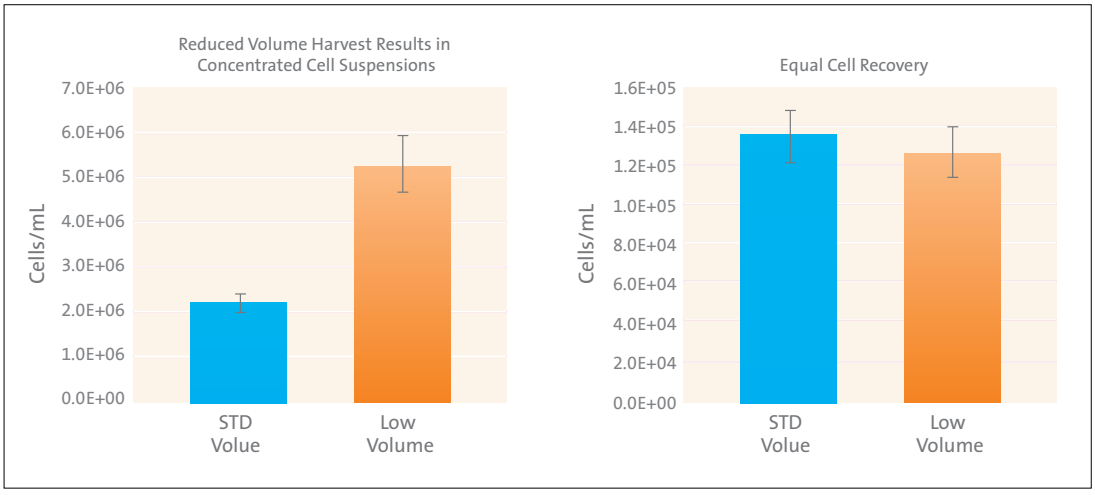
For harvesting cells, use the same dissociating solutions normally used with your cell line when cultured in a T-flask.

1. Remove culture medium as described above.
2. Add 50 to 100 mL rinsing solution (PBS/HBSS or EDTA without Ca^{++} or Mg^{++}).
3. Cap the flask and lay it on its side to allow the solution to distribute evenly between layers (Figure 4 of Instructions for Use manual). This ensures that each layer of the flask receives the same volume of liquid.
4. Rinse.
   a. While the flask is on its side, tilt to a 45° angle toward the cap to pool rinse solution in lower front corner of the flask. This will allow the liquid to collect in the bottom 4-6 layers of the flask.
   b. Once liquid has pooled, rotate the flask back and forth 180° along its long axis several times to thoroughly rinse the bottom layers.
   c. Flip flask over to the opposite side and repeat steps 4a and 4b to rinse top layers.

   Note: For loosely adherent cells such as HEK293 cells, tapping of the flask during the rinse step is sufficient to remove the cells. There is no subsequent need for dissociating reagents. A second PBS rinse could be necessary to recover all cells.

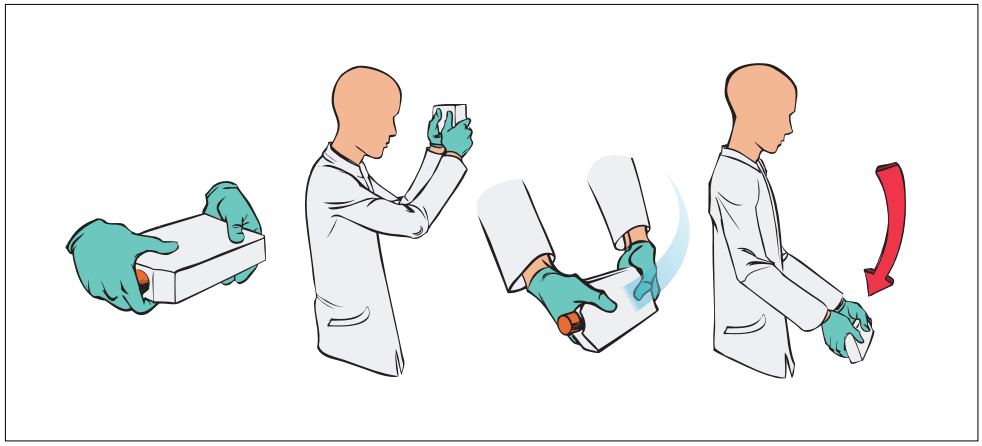
   Note: Before discarding rinse solution, inspect the flask under a microscope to ensure that cells have not detached during handling.
5. Pour or aspirate out the rinsing solution and replace with 50 to 100 mL of dissociation solution.
6. Repeat steps 3 and 4 to evenly expose the cells to the dissociation solution.

   Note: Before discarding dissociation solution, inspect the flask under a microscope to ensure that cells have not detached during handling.
7. Pour or aspirate out the dissociation solution.
8. If desired, place vessel in an incubator to facilitate cell detachment. Closely monitor the cells under the microscope for signs of rounding up of cells.



Corning HYPER*Flask* cell culture vessel low volume vs. standard volume harvest. CHO-K1 cells seeded at 3,000 cell/cm^2 and grown for 96 hours. Trypsin harvest comparing standard (STD) volume harvest (100 mL) vs. low volume harvest (50 mL). Results demonstrate desired concentrated cell suspension (left panel) without loss of cell recovery (right panel). Results are averaged from 12 HYPER*Flask* vessels per condition from four independent studies.

9. Shake, tap or flip the vessel sharply to dislodge the cells by using a gravity flip:
    a. Once cells begin rounding up, place vessel on its side. Firmly grip the neck and the bottom of the flask and raise it to eye level.
    b. In one swift motion, bring vessel down towards your waist while keeping your elbows pressed against your side. Use wrist action half way down to "flick" the vessel downward:



    c. Check under microscope. If cells remain attached, repeat procedure.
10. Once monolayer/cells round up and start to dislodge, add 50 mL of solution to dilute or neutralize dissociation solution.
11. Repeat gravity flip to fully remove any remaining attached cells and to pool cell suspension to one side of the flask.
12. Collect concentrated cell suspension into a suitable collection vessel such as a 50 mL centrifuge tube (Cat. No. 430897).

    Note: It may be necessary to pipette the cell suspension up and down while in the tube to break up any cell clumps.

Optional Rinse Step

If necessary, perform a last rinse by adding 50 to 100 mL of rinsing solution, such as PBS, to the vessel.

13. Repeat harvest steps 3 and 4 or gravity flip.
14. Pool rinse with collected cell suspension.

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