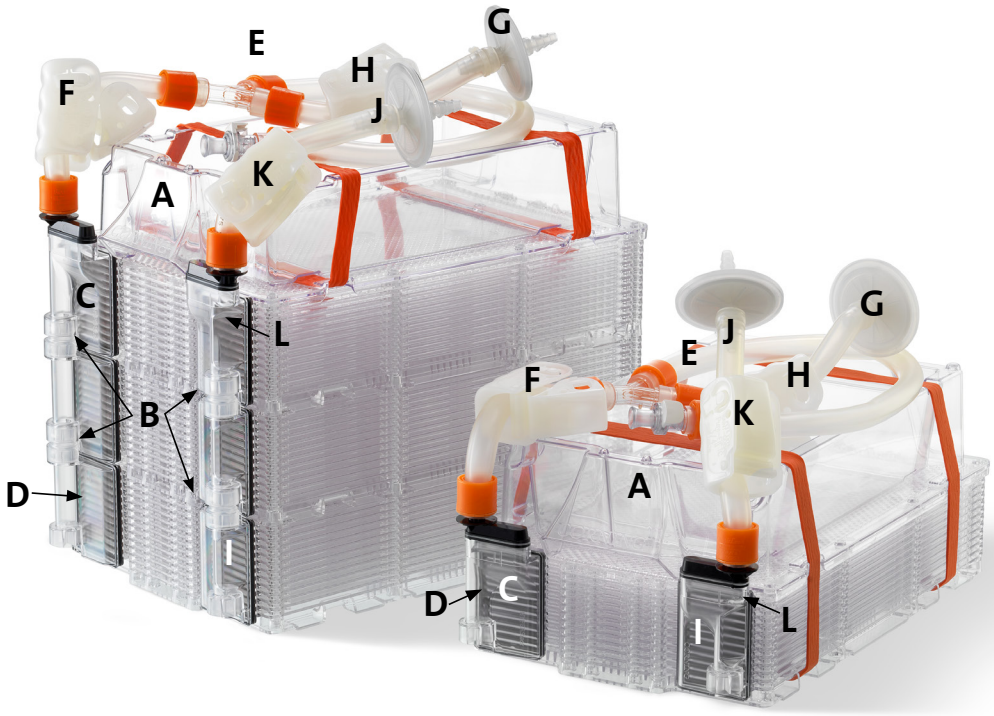
Corning® HYPERStack® Cell Culture Vessels with Single Vent Filter

Guidelines for Use

CORNING

This document is intended to provide basic instructions for using the Corning HYPERStack 12-layer and 36-layer cell culture vessels with a single vent filter. Additional information may be found at **www.corning.com/hyper** or by contacting Scientific Support.

Corning HYPERStack Vessel with Single Vent Filter Components



A. Accessory tray

B. Intermodule connectors (36-layer vessel only)

Liquid Handling Section

C. Liquid handling chambers

D. Volume reference marking lines

E. Liquid handling tubing: 3/8" ID x 5/8" OD with female medical plastic coupler (MPC) and cap

F. Liquid handling tubing clamp

G. Chase tubing: 3/8" ID x 5/8" OD with 0.2 µm chase vent filter

H. Chase tubing clamp

Air Handling Section

I. Air handling chambers

J. Air vent tubing: 3/8" ID x 5/8" OD with 0.2 µm air vent filter

K. Air vent tubing clamp

L. Fill line reference marking

Setting Up the Corning® HYPERStack® Cell Culture Vessel with Single Vent Filter

Remove the Corning HYPERStack vessel from its packaging, and place the provided clamp on the air vent tubing line (Figure 1A).

Close all three clamps on the liquid handling, air vent and chase tubing lines (Figures 1A to 1C). The accessory tray for the 12-layer HYPERStack vessel can be removed if desired.

Aseptically connect the HYPERStack vessel to an inoculum container by using the MPC on the liquid handling tubing (Figure 1D) or via tube welding.

Corning HYPERStack Cell Culture Vessel with Single Vent Filter Manipulation

For best results, Corning HYPERStack vessels need to be held in specific positions and at specific angles when filling, depressurizing, incubating, harvesting, and emptying (Figures 1E to 1J). To achieve these angles, it is recommended to use the Corning HYPERStack Nest Accessory (Cat. No. 10047). The use of the Corning HYPERStack Nest Accessory and alternative methods for achieving similar positions/angles are outlined in this document and in the Corning HYPERStack Nest Guidelines for Use (CLS-AN-622DOC).

HYPERStack vessel on its side with the liquid handling chambers parallel to the working surface and the back of the vessel raised approximately 11 degrees (Figure 1E).

Figure 1E. Initial Fill/Final Empty position.

HYPERStack vessel on its side with the liquid handling chambers in a compound angle position of 8 and 12 degrees (bottom to top and back to front, respectively) to place the air vent filter at the highest point (Figure 1F).

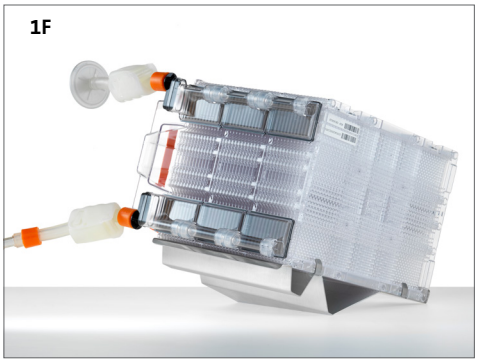


Figure 1F. Final Fill/Initial Empty position.

HYPERStack vessel flat on bottom plate with tubing lines at highest points (Figure 1G).

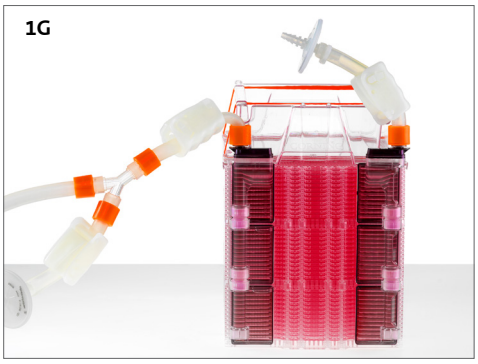


Figure 1G. Incubation/Depressurization position.

During the harvest procedure, the HYPERStack vessel is placed flat on the working surface with the liquid handling side flush to the working surface Figure 1H).

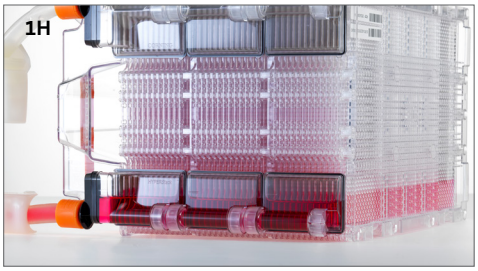


Figure 1H. Equilibration position.

During the harvest procedure to evenly isolate the dissociation reagent in all layers, the HYPERStack vessel is placed flat on its back with the air and liquid handling chambers at the highest position (Figure 1I).

Figure 1I. Isolation position.

Filling the Corning® HYPERStack® Cell Culture Vessel with Single Vent Filter

Corning HYPERStack vessels are meant to be filled completely (1,310 and 3,920 milliliters for 12- and 36-layer vessels, respectively).

NOTE: This can be done via gravity or positive pressure. Roller bottles, Erlenmeyer flasks, and single-use media bag accessories can be used to facilitate closed system liquid handling into the HYPERStack vessels. If other filling methods are desired, please consult with your local Corning Representative or Scientific Support for recommendations and process optimization.

As liquid enters the vessel through the liquid handling side (liquid handling tubing and liquid handling chambers), air escapes through the air handling side (air handling chambers, air vent tubing, and air vent filter). To prevent pressurization of the vessel during use, care must be taken not to block or disrupt air flow from the air vent filter. During liquid movement in and out of the vessel, the air vent tubing clamp must remain open and the air vent filter must remain dry and unobstructed. **CAUTION: Do not overfill the vessel so that liquid reaches the air vent filter and do not use the air vent tubing clamp to control the flow rate.** Obstruction of the air vent filter may cause over-pressurization and over-filling of the vessel, which can lead to structural damage and subsequent leaking from the vessel.

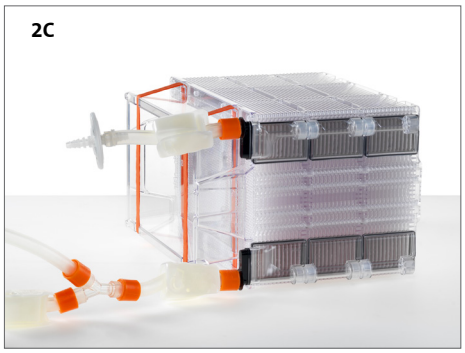
The maximum fill rate is limited by the air vent filter and by cell culture conditions. Filling a HYPERStack vessel at too high a rate may lead to the over-pressurization of the vessel. Excess pressurization may increase shearing conditions leading to cell detachment. Over-pressurization of the vessel can lead to structural damage and subsequent leaking from the vessel. To avoid excess pressurization of the HYPERStack vessel, a flow rate of no more than 2.3L/minute is recommended. When filling by gravity, this flow rate can be achieved by placing the inoculum container no more than 36 inches (91.4 cm) above the working surface.

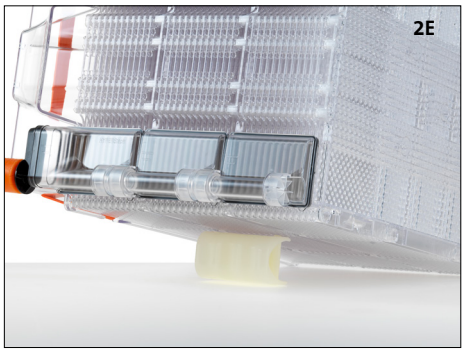
1. Aseptically connect the HYPERStack vessel to an inoculum container using the liquid handling tubing MPC or via tube welding.
2. Load the HYPERStack vessel onto the Corning HYPERStack Nest Accessory following steps outlined in the Corning HYPERStack Nest Guidelines for Use (CLS-AN-622DOC).

3. Bring the HYPERStack vessel to the Initial Fill/Final Empty position by pushing down on the upper corner of the top air-handling chamber (Figure 2A) to rock the Nest forward such that the liquid handling chamber is parallel to the working surface and the back of the vessel is raised approximately 11 degrees (Figure 2B).

   NOTE: If not working with the Nest, place the liquid handling side of the vessel parallel to the working surface (Figure 2C). Elevate the front of the vessel two inches by placing an object such as a tubing clamp under the vessel to achieve approximately a 10-degree angle lift (Figures 2D and 2E).

Figures 2A and 2B. Initial Fill/Final Empty position

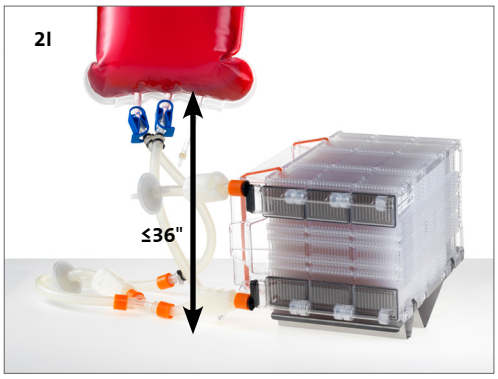




4. Open the air vent tubing clamp first, and then the liquid handling tubing clamp (making sure that the chase tubing clamp remains closed) (Figures 2F and 2G).

5. Initiate liquid movement into the HYPERStack vessel (Figure 2H) from the inoculum container.

   - **Filling by Gravity.** Slowly raise the inoculum container to increase the flow rate of liquid into the HYPERStack vessel. Raising the inoculum container too fast or too high will cause internal pressurization of the vessel. We recommend raising the inoculum container no more than 36 inches (91.4 cm) above the working surface to avoid excess pressurization (Figure 2I).

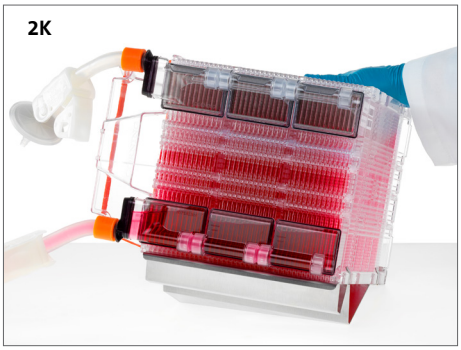


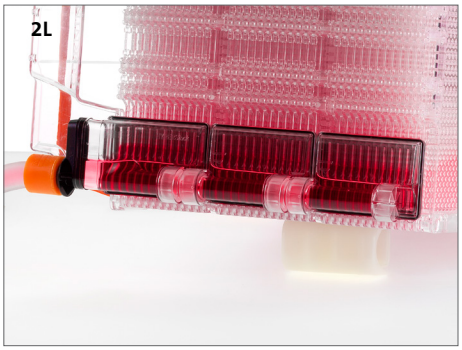
   - **Filling using Positive Pressure.** Aseptic Transfer Cap accessories can be used to facilitate the closed system liquid handling of HYPERStack vessels. Fluid movement into the HYPERStack vessel is achieved through positive pressure on the aseptic transfer cap. The Corning 850 cm² non-treated polystyrene roller bottle (Cat. No. 431644) with Corning Roller bottle disposable tubing set (Cat. No. 10043), or the pre-assembled Corning 2L Erlenmeyer flask with transfer cap (Cat. No. 431518) are recommended for liquid transfer with the 12-layer HYPERStack vessel. The Corning 5L Erlenmeyer flask with Disposable Aseptic Transfer Cap (Cat. No. 11501) is recommended for liquid transfer with the 36-layer HYPERStack vessel.

     - Apply positive pressure (approx. 1.5 pounds per square inch (psi), approx. 10.3 kilopascals [kPa]) to the inoculum container to force liquid to move into the HYPERStack vessel at a flow rate of no more than 2.3L/minute. Positive pressure can be applied using a bench top pump, a fully charged serological pipet controller, or a hand pump.
     - Once liquid movement has been initiated into the HYPERStack vessel, gravity can be used to continue the liquid flow.
     - Periodically apply positive pressure through the aseptic transfer cap vent filter to maintain liquid flow.

6. When liquid enters the air vent chambers (Figure 2J, arrow) bring the HYPERStack vessel to the Final Fill/Initial Empty position by tilting the vessel back. This will raise the front of the Nest placing the vessel in a compound angle position of 8 and 12 degrees (bottom to top and back to front, respectively). The compound angle position protects the air vent filter by placing it at the highest point (Figure 2K).

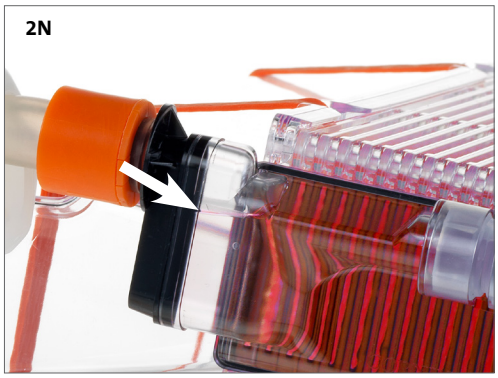
   NOTE: If not working with the Nest, leave the vessel in the 10-degree angle (Figure 2L).





7. To control the flow rate and prevent overfilling of the vessel, slightly squeeze or close the liquid handling tubing clamp when liquid starts to fill the air handling chambers (Figure 2M). This action will allow for air and liquid within the vessel to equilibrate before reaching the “fill line” reference marking. Air/liquid equilibration is reached when liquid levels in all layers stop moving. **CAUTION: Do not use the air vent tubing clamp to control the flow rate. Obstruction of the air vent may cause over-pressurization and over-filling of the vessel, which can lead to structural damage and subsequent leakage.**

8. Bring the liquid level to the “fill-line” reference marking on the top air handling chamber (Figure 2N, arrow).

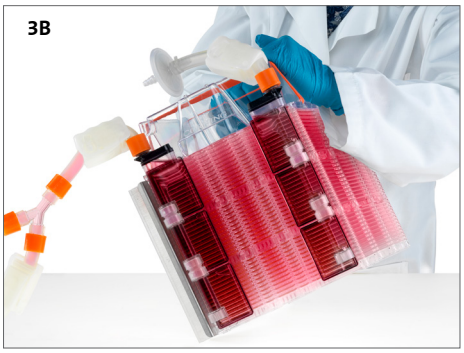


9. Close the liquid handling tubing clamp first (Figure 2O), and then close the air vent tubing clamp (Figure 2P).

Depressurizing the Corning® HYPERStack® Vessel with Single Vent Filter After Filling

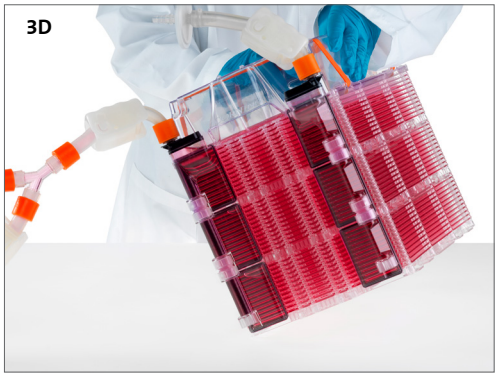
Depressurization is necessary to alleviate any residual pressure introduced into the vessel during filling and to clear the remaining liquid from the liquid handling tubing. Before initiating the depressurization process, make sure the liquid handling tubing, air vent tubing, chase vent tubing, and the inoculum tubing clamps are all closed.

1. Use the Nest handle and the side of the vessel or the Accessory Tray to rotate the HYPERStack vessel from the Final Fill/Initial Empty position to the Incubation/Depressurization position (Figures 3A, 3B, and 3C).



2. The Nest accessory can be unloaded and removed at this time.

   NOTE: If not using a Nest accessory, use the Accessory Tray handle to rotate the vessel into the Incubation position (Figure 3D).



3. To depressurize the vessel:
   - Hold the liquid handling tubing above the media level in the vessel.
   - Open the liquid handling tubing clamp (Figure 3E) to allow liquid remaining in the tubing to flow into the vessel and force any remaining air bubbles out of the vessel.

NOTE: Some side-to-side tilting or back and forth rocking of the vessel may be necessary to force all of the air bubbles out of the vessel.

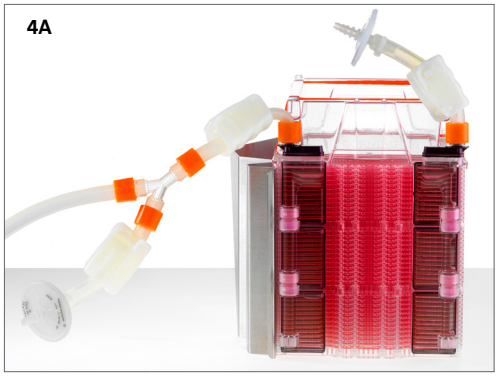
- Close the liquid handling tubing clamp when complete.

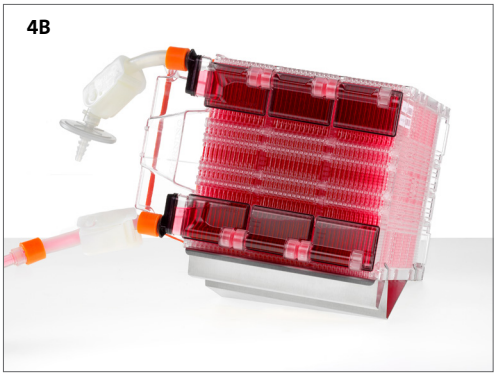
4. To remove residual liquid from the liquid handling tubing:
   - The liquid handling tubing clamp and air vent tubing clamp should remain closed.
   - Lower the inoculum container no more than 36 inches (91.4 cm) below the level of the HYPERStack vessel.
   - Open the inoculum vessel tubing clamp.
   - Hold the chase tubing with chase vent filter above the media level in the HYPERStack vessel, then open the chase tubing clamp. Allow excess media to drain back into the inoculum container. **Option:** Positive pressure can be applied to the chase vent filter to increase the speed of flow.
   - When complete, close the chase tubing clamp then close the inoculum tubing clamp.
5. Aseptically disconnect the HYPERStack vessel from the inoculum container. **Option:** If using a media bag as the inoculum container, the empty bag can remain attached to the HYPERStack vessel during incubation by rolling it up and placing it in the Accessory Tray.
6. Place the HYPERStack vessel into an incubator in the Incubation position with all of the tubing clamps closed.

Emptying the Corning® HYPERStack® Vessel with Single Vent Filter

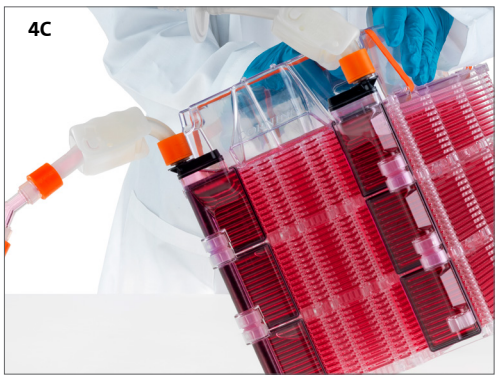
For best results, HYPERStack vessels are emptied using gravity. The maximum empty rate is limited by the air vent filter and by cell culture conditions. Emptying the vessel at too high a rate may produce a vacuum inside the vessel, which can lead to damage or loss of cells as well as structural damage and subsequent leakage from the vessel. The recommended flow rate is no more than 2.3L/minute; this can be achieved by placing the receiving container no more than 36 inches (91.4 cm) below the level of the HYPERStack vessel. The same Aseptic transfer cap accessories referenced for closed system filling can be used to facilitate the closed system emptying of the HYPERStack vessels. Single-use bags (10L or 20L) are recommended when working with larger volumes.

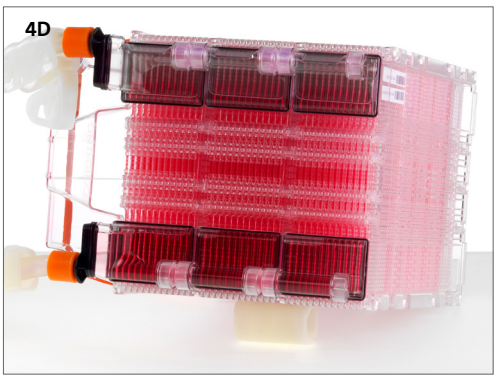
1. Remove the HYPERStack vessel from the incubator and aseptically connect to a receiving/collection container using the liquid handling tubing MPC or by tube welding.
2. Load the HYPERStack vessel onto the HYPERStack Nest accessory (Figure 4A). Turn the vessel to the Final Fill/Initial Empty position by gently tipping the vessel to the side so that the liquid handling side of the vessel is resting on the HYPERStack Nest in the compound angle position of 8 and 12 degrees (bottom to top and back to front, respectively). This position protects the air vent filter by placing it at the highest point (Figure 4B).





NOTE: If not working with the Nest, gently turn the vessel to the side so that the liquid handling side of the vessel is parallel to the working surface (Figure 4C). Bring the front of the vessel up two inches by placing an object such as a clamp under the vessel to achieve a 10-degree angle lift (Figures 4D).





3. Place the receiving container no more than 36 inches (91.4 cm) below the level of the HYPERStack vessel.
4. Open the receiving container tubing clamp, then open the liquid handling tubing clamp on the HYPERStack vessel (Figure 4E).

   NOTE: The liquid handling tubing can remain pinched after the tubing clamp is released. If this occurs, decompress the pinched area of the tubing with your fingers to open the fluid path.
5. Once liquid starts flowing from the vessel into the receiving container, open the air vent tubing clamp (Figure 4F). **CAUTION: The air vent tubing clamp should be opened after the liquid handling tubing clamp in order to prevent liquid from rushing into the air vent tubing and wetting-out the filter.**

6. To control the flow rate, lift or lower the receiving container or squeeze the liquid handling tubing clamp. **CAUTION: Do not use the air vent tubing clamp to control the flow rate of liquid out of the vessel.** Obstructing the air vent may produce a vacuum inside the vessel, which can lead to damage or loss of cells and structural damage and subsequent leakage from the vessel.
7. When the HYPERStack vessel is approximately half empty, push the front of the vessel down (Figure 4G) to pivot the Nest into the Initial Fill/Final Empty position. In this position the liquid handling side is parallel to the working surface and the back of the vessel is raised to an 11-degree angle (back end 3.2 inches (8 cm) higher than the front end) (Figure 4H).

   NOTE: If not working with the Nest, raise the back end of the vessel up 10-degrees (back end 2 inches (5 cm) higher than the front end) by placing an object such as a clamp under it. (Figure 4I)

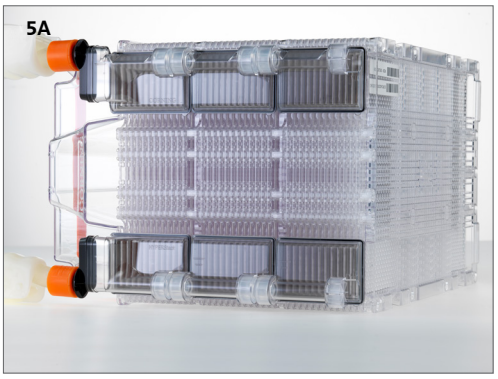
8. To drain the HYPERStack vessel completely:
   - Tilt the back of the HYPERStack vessel up and pivot the front end of the Nest forward to guide the residual liquid towards the liquid handling tubing.
   - The HYPERStack vessel and BarbLock® retainer on the liquid handling tubing should not come in contact with the working surface. The Nest accessory should be bearing the weight of the HYPERStack vessel.
   - If not working with the Nest, pick up the back of the vessel and tilt forward to guide the residual liquid towards the liquid handling tubing.
9. Once the vessel is fully drained, close the liquid handling and air vent tubing clamps.

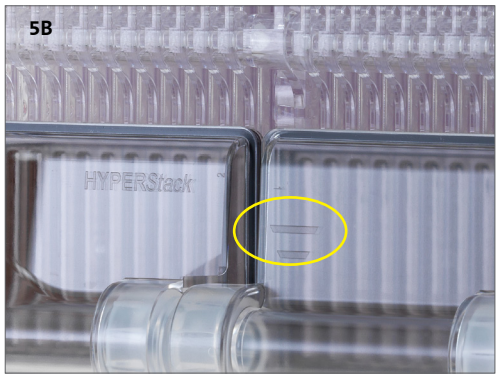
10. To drain residual liquid from the liquid handling tubing to the receiving/collection container:
    - Lift the chase tubing and chase vent filter and open the chase tubing clamp. It may be necessary to decompress any pinched portion of the chase tubing to initiate liquid movement.
    - After all liquid has been transferred to the receiving/collection container, close the chase tubing and receiving container tubing clamps.
11. The vessel can be aseptically disconnected from receiving container.

Harvesting Adherent Cells from the Corning® HYPERStack® Vessel with Single Vent Filter

The same harvesting steps and reagents used for harvesting cells from a standard cell culture vessel can be used to harvest cells from HYPERStack vessels. The minimum recommended reagent volumes for harvesting are 200 mL for the 12-layer vessel and 600 mL for the 36-layer vessel, but ideal harvest volumes should be determined empirically depending on the specific application, cell type, and harvest reagent type. Harvest may be improved by increasing reagent volumes. Harvest reagents may be pre-warmed prior to harvest to achieve optimal dissociation time.

When the vessel is in the Equilibration position—flat on the working surface with the liquid handling side parallel to the working surface (Figure 5A)—reference markings on the liquid handling chambers approximate the minimum recommended reagent volumes. The lower markings are approximately 200 mL in the 12-layer vessel and 600 mL in the 36-layer vessel (Figure 5B).



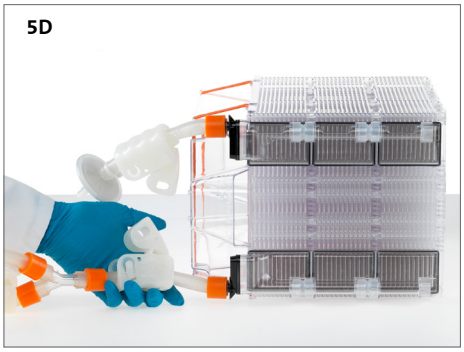


Most cell lines detach faster from the HYPERStack polystyrene film compared to a standard flask surface. The liquid and air handling chambers can be used to visualize the turbidity of the dissociation as an indicator of cell detachment. Harvest optimization is recommended using the Corning HYPERFlask® vessel prior to harvesting with a HYPERStack vessel.

The same Aseptic Transfer Cap accessories referenced for filling and emptying HYPERStack vessels can be used to facilitate the closed system liquid handling of the HYPERStack vessels during harvest.

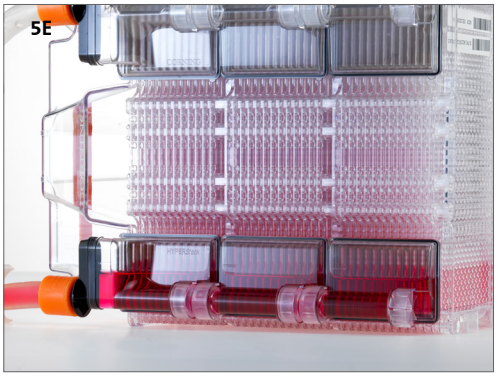
1. Completely remove spent media from the HYPERStack vessel.
2. Pre-fill a harvest container with dissociation reagent and a collection container with quench solution.

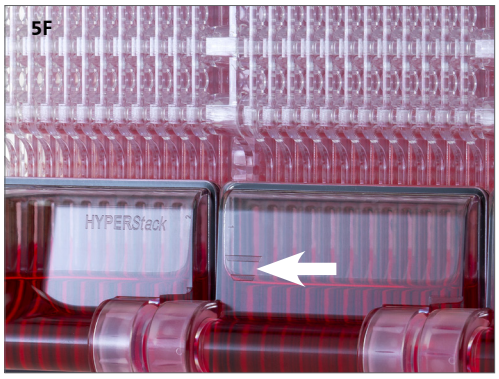
   NOTE: It is recommended that the quench solution volume equals the dissociation solution volume.
3. Aseptically connect the harvest container to the liquid handling tubing of the HYPERStack vessel.
4. Place the HYPERStack vessel in the Equilibration position--vessel flat on its side with the liquid handling side parallel to the work surface (Figure 5A).
5. Open the air vent and harvest container tubing clamps, then the liquid handling tubing clamp. (Figures 5C and 5D).



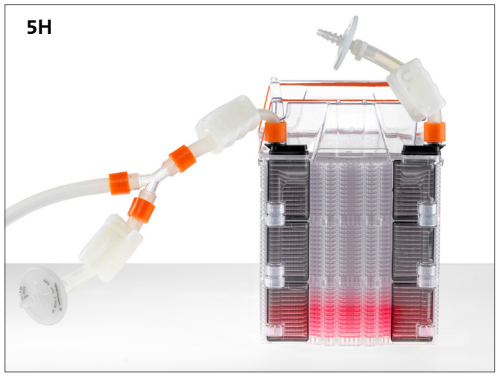
6. Following the recommendations in the Filling the Corning® HYPERStack® Cell Culture Vessel section of this document, apply positive pressure to the harvest container aseptic transfer cap assembly or initiate movement of the dissociation reagent into the HYPERStack vessel via gravity.
   - Positive pressure can be applied using a bench top pump, a fully charged serological pipet controller, or a hand pump at approximately 1.5 pounds per square inch (psi), or approximately 10.3 kilopascals [kPa]) through the 0.2 µm vent filter on an aseptic transfer cap on the harvest container. This will pressurize the container and force the dissociation reagent to move from the container into the HYPERStack vessel.
   - A flow rate of no more than 2.3L/minute is recommended when transferring liquid into the vessel by raising the harvest container no more than 36 inches (91.4 cm) above the working surface.
7. Once the desired volume of dissociation reagent has entered the HYPERStack vessel, close the liquid handling tubing, harvest container tubing, and air vent tubing clamps.

   NOTE: When working with the 36-layer vessel, the lower reference mark on the bottom liquid handling chamber (Figure 5E) indicates approximately 600 mL volume level (Figure 5F).





8. Evenly distribute the harvest solution throughout the layers:
   - Turn the HYPERStack vessel to the Isolation position to evenly retain the dissociation reagent on all layers (Figure 5G). Next, lower the vessel to the Incubation position (Figure 5H).



- Use the Accessory Tray to gently rock the HYPERStack vessel towards and away from you along the long edges of the vessel (Figures 5I and 5J). Distribute the harvest solution in this manner for at least five complete cycles.

NOTE: Limit the front to back rocking of the vessel to evenly retain the dissociation reagent on all layers.

- Depending on the cell type, harvest reagent solution and volume, Step 9 may need to be repeated several times.

9. Proceed with dissociation incubation:
   - Rotate the HYPERStack vessel to the Equilibration position to evenly distribute the dissociation solution to all layers.
   - Turn the vessel to the Isolation position, then lower to the Incubation position.
   - Distribute the dissociation solution evenly as indicated in Step 9.
   - Leave the vessel in the Incubation position for the desired time to achieve cell detachment.
10. Once dissociation is complete, turn the HYPERStack vessel to the Equilibration position and prepare to add quench solution into the HYPERStack vessel to neutralize the harvest reagent and collect cell suspension.

    NOTE: Alternatively, the harvest solution can be emptied directly into a collection/quench container containing quench solution.
11. Aseptically disconnect the harvest container from the HYPERStack vessel then aseptically connect the collection/quench container (pre-filled with quench solution).
12. Open the air vent tubing and collection/quench container tubing, and then the liquid handling tubing clamps.
13. Following the recommendations in the Filling the Corning HYPERStack Cell Culture Vessel section of this document, apply positive pressure to the collection/quench container aseptic transfer cap assembly or initiate movement of the quench solution into the HYPERStack vessel via gravity.
14. Once the desired volume of quench solution is transferred into the HYPERStack vessel, close the liquid handling, collection/quench container tubing, and air vent tubing clamps.
15. Turn the HYPERStack vessel to the Isolation position, then lower to the Incubation position. Gently distribute the quench solution by rocking the vessel side to side along the long edges of the vessel (Step 9) to evenly distribute the quench solution and completely neutralize the cell dissociation reagent.
16. Move the HYPERStack vessel to the Equilibration position. Place the collection/quench container no more than 36 inches (91.4 cm) below the level of the HYPERStack vessel.
17. Open the air vent tubing and collection/quench container tubing clamps, then the liquid handling tubing clamp to drain the neutralized cell suspension into the collection container.

    NOTE: Repeating Steps 12 through 18 with quench solution or collection solution may be desired to collect all cells dissociated during harvest.
18. When all dissociated cells have been collected in the collection/quench container, close the collection/quench container tubing clamp and liquid handling tubing clamp, then aseptically disconnect from the HYPERStack vessel. The cell suspension is ready for processing.

Specifications

Corning® HYPERStack® Vessel with Single Vent Filter	12-layer	36-layer
Cat. No.	20012 20013	20036 20037
Qty/Pk	1	1
Qty/Cs	4	2
Growth Area (cm²)	6,000	18,000
Fill Volume (mL)	1,310	3,920
Weight Empty	5 lbs. (2.2 kg)	8.7 lbs. (6.6 kg)
Weight Filled	7.8 lbs. (3.5 kg)	23.5 lbs. (10.5 kg)
Minimal Reagent Harvest Volume	200 mL	600 mL
Maximum Flow Rate	2.3L/minute	2.3L/minute

References

Corning HYPERStack Nest Accessory Guidelines for Use (CLS-AN-622DOC).
Corning HYPERStack Cell Culture Vessels Frequently Asked Questions (CLS-AN-749).

How to Purchase: For specific availability in your region and purchasing options, terms and conditions of sale, customer/product support, and certificates, visit **www.corning.com/how-to-buy**.

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