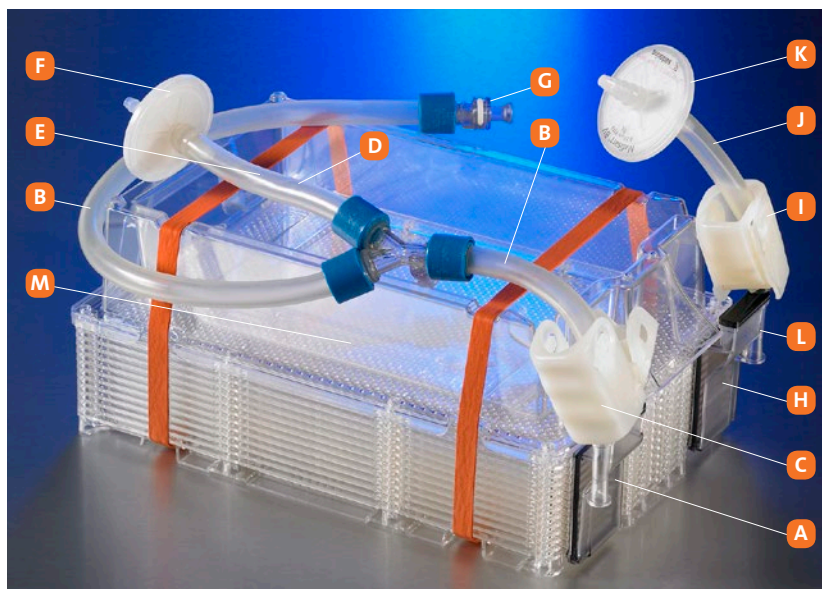


Corning® HYPERStack® Cell Culture Vessel Closed System

User Guide

CORNING

Corning HYPERStack Vessel Components



Liquid Handling Section

- A Liquid handling manifold
- B 3/8" I.D. liquid handling tubing
- C Liquid handling tubing clamp
- D Chase tubing
- E Chase tubing clamp location
- F 0.2 μ m chase vent filter
- G Female MPC quick-connect coupler with cap

Air Handling Section

- H Air handling manifold
- I Air vent tubing clamp
- J Air vent tubing
- K 0.2 μ m air vent filter
- L Fill line
- M Stacking tray

This protocol can be used with either the Corning HYPERStack 12-layer or HYPERStack 36-layer cell culture vessel. It is intended as an initial guide to using these vessels. It is highly recommended that you also view the User Guide video on the Corning Life Sciences YouTube channel. For manifolding of multiple vessels, contact your Corning Bioprocess Specialist.

Setting Up the Corning HYPERStack Vessel



Remove the vessel from its packaging and inspect it for damage. Place a clamp on the air vent tube at the middle of the tubing or 2/3 away from HYPERStack port (some models will already have this clamp in place). Close all three clamps (Figures 1A - 1C). The storage/stacking tray for the 12-layer vessel can be removed if desired. Readjusting the tie wraps is not needed and can affect the sterilized tubing.

Prepare the cell inoculum in 1.3L for 12-layer vessel or 3.9L for 36-layer vessel.

- ▶ 12-layer vessel provides 6,000 cm² of growth area.
- ▶ 36-layer vessel provides 18,000 cm² of growth area.

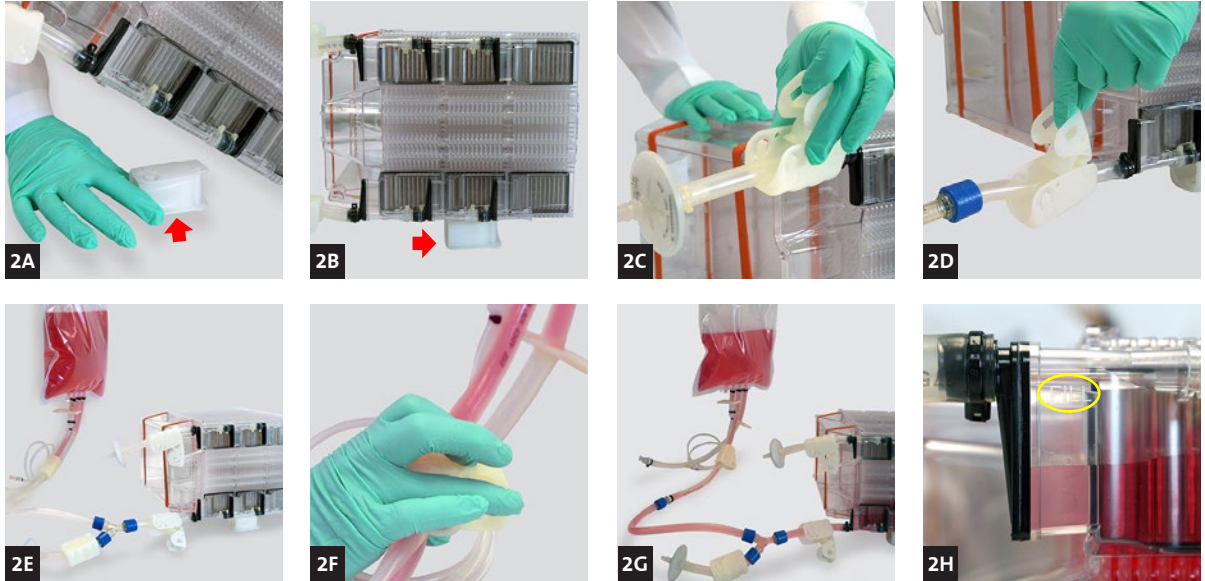
Connect the HYPERStack vessel to the inoculum container by tube welding or by using the MPC quick-connect coupler under aseptic conditions.

Seeding/Filling the Corning® HYPERStack® Vessel

Corning HYPERStack cell culture vessels are meant to be filled completely to the “Fill” line as indicated on the side of the vessel. This can be done by gravity, peristaltic pump, or positive pressure. As liquid enters the vessel through the liquid handling tubing, manifold air escapes through the air vent filter. To prevent pressurization of the vessel during use, care must be taken not to block air flow from the vent filter or to overfill the vessel, which will wet the filter and disrupt air escape. During liquid movement in and out of the vessel, the air vent tubing clamp **must** remain open. Failure to do so can cause structural damage to the vessel and subsequent leakage may occur due to internal pressure.

Select one of the three methods below to seed or fill your HYPERStack vessel.

Gravity Method



1. Position the HYPERStack vessel to “Fill” by placing the liquid handling side of the vessel on the working surface with the front of vessel at a 10 degree angle (front end 2 in./5 cm higher than the back end). This can be accomplished by placing a clamp under the vessel (Figures 2A and 2B).
2. Connect the inoculum container to the HYPERStack vessel.
3. Open the air vent and liquid handling tubing clamps on the HYPERStack vessel (Figures 2C and 2D). The chase tubing clamp must remain closed at this point. The completed setup is shown in Figure 2E. Open the clamp for the inoculum container to initiate liquid flow (Figure 2F).
4. Slowly raise the inoculum container to increase the flow rate (Figure 2G). Raising the inoculum container too fast or too high will cause internal pressurization of the HYPERStack vessel. We recommend keeping the inoculum container no more than approximately 2 in./5 cm higher than the HYPERStack vessel.
5. Liquid flow rate can be controlled by gently squeezing the liquid handling tubing clamp.
6. Slowly bring the liquid level to just below the “Fill” line (Figure 2H) on the air handling manifold, and then quickly close the liquid handling clamp followed by the air vent clamp. To control the flow rate and prevent overfilling or wetting-out the vent filter, you can either lower the inoculum container to just below the “Fill” line on the air manifold or squeeze the liquid handling tubing clamp.

Do not use the air vent tubing clamp to control the flow rate. Obstructing the air vent tubing may cause over-pressurization and over-filling of the vessel, which can lead to structural damage and subsequent leakage.

Peristaltic Pump Method

Aseptically connect the Corning® HYPERStack® vessel and the inoculum container to suitable pump tubing. The chemically resistant, heat-sealable flexible tubing is not designed to be used with a peristaltic pump.

1. Position the HYPERStack vessel to “Fill” by placing the liquid handling side of the vessel on the working surface with the front of vessel at a 10 degree angle (front end 2 in./5 cm higher than the back end). This can be accomplished by placing a clamp under the vessel (Figures 2A and 2B).
2. Position the pump tubing in the peristaltic pump, ensuring the flow direction is toward the vessel.
3. Set the pump speed to 0.5 liters/minute. Open the air vent, as well as the liquid handling and inoculum tubing clamps (Figures 2C - 2D). The chase tubing clamp must remain closed.
4. Start the liquid flow at a low rate (~0.5 liters/minute) to initiate liquid movement. After liquid transfer is initiated without issue, the pump fill rate can be increased to a maximum fill rate of 1.9 liters/minute. This maximum fill rate is limited by the vent filter to a maximum flow rate of approximately 2.0 liters/minute.
5. When the vessel is about 3/4 full, slow the pump down to 0.5 liters/minute to prevent overfilling and accidental wetting of the air filter.
6. Bring the liquid level to just below the “Fill” line on the air handling manifold (Figure 2H). Quickly stop the peristaltic pump, close the liquid handling clamp, and stop the peristaltic pump. **Use the peristaltic pump to control the flow rate into the vessel.**

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.

Positive Pressure Method

Aseptic Transfer Cap accessories can be used to facilitate the closed system liquid handling of the HYPERStack vessels (*Corning Roller Bottle Aseptic Transfer Cap User Guide*, CLS-AN-198). Fluid movement is achieved through positive or negative pressure to fill or empty the vessel. The Corning roller bottle aseptic transfer cap (Corning Cat. No. 10043) is recommended for use with the 12-layer vessel. The Corning 5L Erlenmeyer flask aseptic transfer cap (Corning Cat. No. 11500) can be used with the 36-layer vessel.

1. Position the HYPERStack vessel to “Fill” by placing the liquid handling side of the vessel on the working surface; the front of vessel up to a 10 degree angle (front end 2 in./5 cm higher than the back end). This can be accomplished by placing a clamp under the vessel (Figures 2A and 2B).
2. Set the pump speed to 0.5 liters/minute. Open the air vent, as well as the liquid handling and inoculum tubing clamps (Figures 2C - 2D). The chase tubing clamp must remain closed.
3. Apply positive pressure (~1.5 pounds per square inch (psi), ~10.3 kilopascals [kPa]) through the 0.2 µm vent filter on the aseptic transfer cap. This will pressurize the inoculum container and force liquid to move from the inoculum container into the HYPERStack vessel. Positive pressure can be applied using a bench top pump, a fully charged pipet controller, or a hand pump.
4. Once liquid movement has been initiated into the HYPERStack vessel, gravity can be used to continue the liquid flow.
5. Periodically apply positive pressure to the aseptic transfer cap to maintain liquid flow.

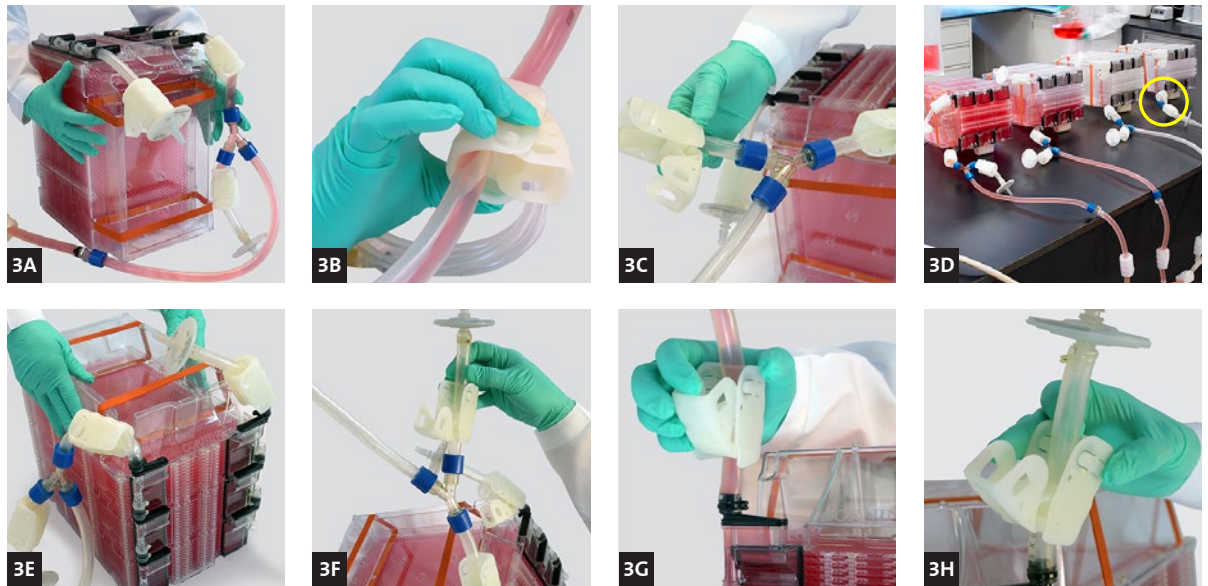
To control the flow rate and prevent overfilling of the vessel, slightly squeeze or close the liquid handling tubing clamp when the vessel is about 3/4 full. This action will allow for air and liquid within the vessel to equilibrate before reaching the “Fill” line (Figure 2H). Air and liquid equilibration is reached when liquid levels in all layers stop moving.

To control the flow rate and prevent over-filling or wetting out the vent filter, you can squeeze the liquid handling tubing clamp.

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.

Equilibrating the Corning® HYPERStack® Vessel After Filling

The equilibration step is used to distribute liquid and air equally throughout the HYPERStack cell culture vessel and to clear remaining liquid from the liquid handling tubing. Before placing the vessel in the equilibration position, make sure the liquid handling tubing, air vent tubing, and the inoculum tubing clamps are all closed.



1. Place the HYPERStack vessel into the “equilibration” position by raising the front of the manifold to the highest point (Figure 3A).
2. Place the inoculum container in a position lower than the media level in the HYPERStack vessel. Open the inoculum tubing clamp (Figure 3B).
3. Hold the chase filter tubing above the media level in the vessel. Open the chase tubing clamp (Figure 3C). Allow excess media to drain back into the inoculum container. Positive pressure can be applied to the chase filter to start the flow, if desired. If using a peristaltic pump, reverse the direction of the media towards the source. If multiple HYPERStack vessels are connected together, only open the chase tubing clamp furthest away from the pump (Figure 3D).
4. Bring the HYPERStack vessel to the incubation position (Figure 3E).
5. Close the chase tubing, liquid handling tubing, and air vent tubing clamps (Figures 3F - 3H).
6. Disconnect the HYPERStack vessel from the inoculum container under aseptic conditions.
Option: If using media bags, the empty bag can remain attached to the HYPERStack vessel during incubation by rolling it up and placing it in the storage tray.
7. Place the HYPERStack vessel into an incubator.

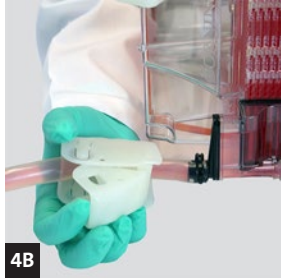
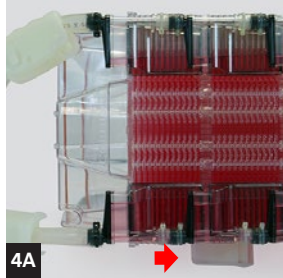
Emptying the Corning® HYPERStack® Vessel

Remove the HYPERStack vessel from the incubator and aseptically connect the HYPERStack vessel to a receiving container by the tube welding or by using the MPC quick-connect coupler.

Place the HYPERStack vessel in the “Fill” position with the front of the vessel elevated at a 10 degree angle (front end 2 in./5 cm higher than the back end). This can be accomplished by placing a clamp under the vessel (Figure 4A).

Next, select one of the two methods listed below to empty the HYPERStack vessel.

Gravity Method



1. Lower the receiving container just below the level of the HYPERStack vessel.
2. Open the receiving container's tubing clamp then the HYPERStack vessel's liquid handling tubing clamp (Figure 4B). Once liquid starts flowing from the vessel into the receiving container open the air vent tubing clamp. It is important to keep in mind that the chemically resistant, heat-sealable flexing tubing can pinch under the clamp during incubation of the HYPERStack vessels, preventing any liquid or slowing liquid movement. If this occurs, slide the open clamp and de-pinch the chemically resistant, heat-sealable flexing tubing with your fingers.
Note: The air vent tubing clamp should be opened last in order to prevent liquid from rushing into the tube and wetting-out the filter.
3. The correct rate to empty the HYPERStack vessel is approximately one liter/minute (1L/min.). To control the flow rate, lift or lower the receiving container or squeeze the liquid handling tubing clamp.
Do not use the air vent tubing clamp to control the flow rate. Obstructing the air vent may cause over-pressurization and over-filling of the vessel, which can lead to structural damage and subsequent leakage.
4. When the vessel is approximately half empty, move the HYPERStack vessel to the “drain” position by raising the back end of the vessel to a 10 degree angle (back end 2 in./5 cm higher than the front end) (Figure 4C) and tilting it slightly forward. This can be accomplished by placing a clamp under the vessel.
5. Once all liquid has drained from the vessel, lift the chase tubing and open the chase tubing clamp to drain any liquid remaining in the liquid handling tubing into the receiving container.
6. Close the liquid handling tubing, the chase tubing, and receiving container tubing clamps.
7. Aseptically disconnect the Corning HYPERStack vessel from the receiving container.

Peristaltic Pump Method

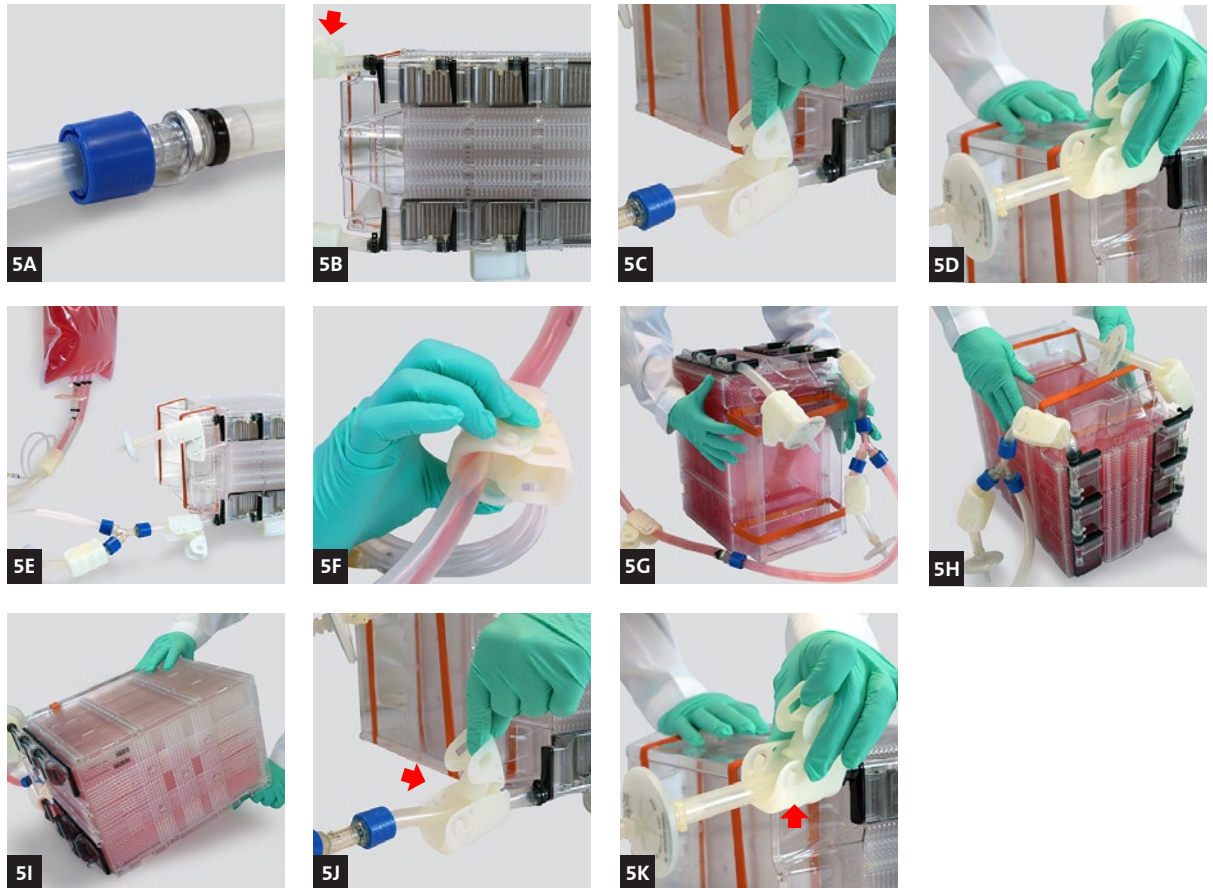
The Corning HYPERStack vessel and the receiving container need to be connected aseptically to suitable pump tubing.

1. Place the HYPERStack vessel in the “Fill” position by placing the liquid handling section flat on the working surface and raising the front of the vessel to a 10 degree angle (front end 2 in./5 cm higher than the back end) (Figure 4A).
2. Position the pump tubing in the peristaltic pump, and ensure that the flow direction is set away from the vessel. Open the liquid handling tubing and receiving vessel tubing clamps.
3. Start the liquid transfer at ~0.5 liters/minute before opening the air vent tubing clamp. The flow rate can then be increased to maximum of 1.9 liters/minute.
4. When the vessel is half empty, move the HYPERStack vessel to the “drain” position by raising the back end of the vessel to a 10 degree angle (back end 2 in./5 cm higher than the front end) (Figure 4C) and tilting it slightly forward. This can be accomplished by placing a clamp under the vessel.
5. After all liquid has been transferred to the receiving container, stop the pump and close the liquid handling tubing, air vent tubing, and receiving container tubing clamps.
6. Aseptically disconnect vessel from receiving container.

Harvesting Adherent Cells from the Corning® HYPERStack® Vessel

The same harvesting steps and reagents used for harvesting cells from a standard cell culture vessel can be used to harvest cells from the 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. The harvest can be performed using roller bottles (*Corning Roller Bottle Aseptic Transfer Cap User Guide, CLS-AN-198*) or by closed system harvest using pre-assembled, single-use bags especially made for the HYPERStack vessel.

This protocol describes a closed system harvest using single-use bags.



1. Aseptically connect the pre-filled trypsin and quench bags by tube welding or by using the MPC quick-connect coupler to create the harvest bag assembly.
2. Aseptically attach the harvest bag assembly to the HYPERStack vessel by tube welding or by using the MPC quick-connect coupler (Figure 5A). Close the harvest assembly tubing clamps.
3. Place the HYPERStack vessel in the “Fill” position (Figure 5B). Open the liquid handling tubing and air vent tubing clamps (Figures 5C and 5D).
4. Raise the harvest bag assembly above the height of HYPERStack vessel (Figure 5E).
5. Open the trypsin and harvest bag assembly tubing clamps (Figure 5F) to add the cell dissociation solution to the vessel.
6. Once the entire dissociation reagent is in the vessel, close the liquid handling, air vent, and trypsin bag tubing clamps.
7. First turn the HYPERStack vessel to the “equilibration” position then place in the incubation position (Figure 5G). Gently rock the vessel side to side and back and forth to distribute the cell dissociation solution over all the layers in the vessel (Figure 5H).

Note: Most cell lines detach faster from polystyrene film compared to a standard flask. Use manifold windows to visualize the dissociation reagent’s turbidity as an indicator of cell detachment. Harvest optimization is recommended using the Corning HYPERFlask® vessel prior to harvesting with a HYPERStack vessel.

8. After incubating the Corning® *HYPERStack*® vessel for the time required to release the cells, place the *HYPERStack* vessel in the “Fill” position again.
9. Place the harvest bag assembly higher than the *HYPERStack* vessel. Open the air vent and liquid handling tubing clamps.
10. Open the quench bag tubing clamp. The quench solution will enter the *HYPERStack* vessel.
11. Close the air vent and the liquid handling tubing clamps. Turn the *HYPERStack* vessel to the “equilibration” position then place in the incubation position. Gently distribute the quench solution by rocking the vessel side to side and front to back to completely neutralize the cell dissociation reagent (Figures 5G and 5H).
12. Place the harvest bag assembly lower than the *HYPERStack* vessel.
13. Place the vessel in the empty/drain position by raising the back end of the vessel to a 10 degree angle (back end 2 in./5 cm higher than the front end) and tilting it slightly forward (Figure 5I). This can be accomplished by placing a clamp under the vessel.
14. Open the air vent and the liquid handling tubing clamps (Figures 5J and 5K).
15. Transfer the cell suspension into the empty quench bag.
16. Aseptically disconnect the harvest bag assembly from the *HYPERStack* vessel. The cell suspension is ready for processing.

General Product Information

- ▶ Growth area per layer: 500 cm²
- ▶ Media per layer: 100 mL
- ▶ Media per cm²: 0.22 mL
- ▶ Stackette internal height: 2.0 mm
- ▶ Triple-bagged
- ▶ Corning CellBIND® surface treatment or non-treated
- ▶ Sterile
- ▶ Size/fill volume (12-layer): 6,000 cm²/1.3L
- ▶ Size/fill volume (36-layer): 18,000 cm²/3.9L
- ▶ Suggested reagent volume* (12-layer): 200 mL
- ▶ Suggested reagent volume* (36-layer): 600 mL

*Cell dissociation solution and buffers.

Ordering Information

Corning® HYPERStack® Cell Culture Vessels

Cat. No.	Description	Qty/Pk	Qty/Cs
10012	HYPERStack 12-layer cell culture vessel, Corning CellBIND surface	1	4
10013	HYPERStack 12-layer cell culture vessel, not treated surface	1	4
10036	HYPERStack 36-layer cell culture vessel, Corning CellBIND surface	1	2
10037	HYPERStack 36-layer cell culture vessel, not treated surface	1	4

Single-use Bags for Corning HYPERStack Cell Culture Vessels

91-200-75	Trypsin bag, ULDPE, 5L	1	1
91-200-76	Quench bag, ULDPE, 5L	1	1
91-200-77	Media bag, ULDPE, 20L	1	1

Accessories

10042	Disposable tubing set for use with glass bottle, 3/8" ID x 1/2" OD, animal component-free, chemically resistant, heat-sealable flexible tubing, 18" in. length, sterile	1	2
10043	Disposable tubing set for use with 850 cm ² polystyrene roller bottle, 3/8" ID x 1/2" OD, animal component-free, chemically resistant, heat-sealable flexible tubing, 0.2 µm filter, MPC quick-connect	1	2
431644	Corning 850 cm ² polystyrene bottle, easy grip cap, not treated, sterile	1	40

MPC = medical plastic coupler.

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

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