Corning® E-Cube™ Culture System
Instruction Manual

Introduction

Welcome to the E-Cube Culture System, Corning’s unique system for growing large quantities of adherent cells. The E-Cube system is easy to use for the evaluation of perfused parallel-plate growth technology. The E-Cube system is designed to help you determine if the larger CellCube® system is the right tool for your application, not to replace a larger and automated standard CellCube system. Rather, it is meant to complement the larger system, acting as a bridge between flasks or roller bottles and the CellCube system.

The uses of the E-Cube system are varied. Its primary purpose is to provide a simple method to assess how well a cell line will grow in CellCube modules prior to investing in the resources and funding that would be necessary for the larger CellCube system. It can also be used to begin process development, since cell behavior in the E-Cube system is similar to that in the larger automated CellCube systems.
Finally, the unit will introduce many of the concepts of bioreactor methodology that have direct relevance to operating the larger system. This includes making sterile connections, inoculating the CellCube® module, and monitoring metabolite utilization by cells in a CellCube system.

The E-Cube™ system provides a base-line level of productivity. This is because manipulations must be made manually, oxygen is only augmented and not controlled, and the system works in a fed-batch manner rather than in true perfusion. In most cases, this means that productivity will only improve when the switch to the automated system is accomplished. Above all, once the system is operating, please review the note (page 12) regarding “Staying ahead of the process”. We find that the biggest factor in these systems underperforming is the lack of early intervention. This applies to the E-Cube system as well as the larger CellCube systems.

We hope that your efforts with the E-Cube system are successful. Should you have any questions, please call Corning’s Technical Services at 1-800-492-1110. Outside the United States call 978-635-2200.

E-Cube Assembly and Operation

Additional Required Materials

1. Two sterile 1 L inoculation vessels, glass or plastic, having caps with two ports as described in Assembly Step 25; see Figure 17 on page 10. Additional 2 L bottles (at least 3) are required for harvesting cells.
2. 2 L cell culture medium to set up the E-Cube system, more will be required for feeding or harvesting the cells.
3. Cable tie gun, used to securely tighten nylon cable ties during assembly.
4. One or two pairs of hemostats, or other pinch clamps, that can be rapidly moved onto and off of the E-Cube silicone tubing
5. Autoclave paper or aluminum foil cut into 15 cm or 6 inch square pieces
6. Spray bottle of 70% ethanol
7. Autoclave tape and autoclave bag at least 30 cm wide by 45 cm long (12 inches wide by 18 inches long)
8. Sterile T-25 cell culture flasks
9. Peristaltic pump, capable of delivering between 0 to 150 mL/minute in fluid volume
10. Sterile pipettes
11. Vacuum pump for aspirating medium
12. Kits or assays for monitoring glucose, lactate and other desired metabolites in the cell culture medium
13. CO₂ incubator large enough to hold the entire E-Cube system and a peristaltic pump
14. Autoclave large enough to hold an E-Cube system without the CellCube module and pump
15. Tools for measuring and cutting tubing and nylon tie wrap
16. 1/8 inch Allen wrench for tightening tubing support posts

Make sure that your peristaltic pump is designed for used in a humidified CO₂ incubator and it does not significantly raise the temperature inside the incubator when it is running. Check the incubator temperature after at least 24 hours of operation.
I. Assembly of the System for Autoclaving

1. Remove the E-Cube™ stand from its packaging. Make sure the bolts on the posts and handles have not loosened during shipment. Use a 1/8 inch Allen wrench to tighten any loose bolts or support posts. Add an O-ring to each of the three short Medium Reservoir support posts (Figure 2).

2. Remove the silicone rubber tubing from its box and cut off one 7.62 m (25 foot) section for use as the Oxygenator Coil; one 61 cm (24 inch) piece for connecting the Cell Seeding Ports to the Medium Reservoir; and one 5 cm (2 inch) and six 15 cm (6 inch) pieces for the remainder of the connections.

3. Wrap the 7.62 m Oxygenator Coil tubing around the four corrugated posts, starting at the bottom groove on each post and working up. Be sure to leave 45 cm (18 inches) free at each end to attach later to the Medium Reservoir and CellCube® module (Figure 2).

4. Remove the glass spinner flask and center cap from its packaging and assemble. This unit will be used as the Medium Reservoir. Optional: if stirring the culture medium in the Medium Reservoir is desired, the impeller assembly should be added to the flask and the unit placed on a slow speed magnetic stirrer during use. Insert the assembled impeller into the cap for the flask, and then place the assembly into the flask and screw on the cap until it is secure (Figure 3). Refer to the assembly directions that come with the Spinner flask for detailed instructions.

5. Two types of sidearm fittings are packaged with the E-Cube system. They are shown in Figure 4. The dual-style sidearm fitting has two long angled stainless steel tubes that extend down to the bottom of the spinner flask. One tube on this fitting will be used to circulate medium, and the other will be fitted with a septum and used as a Sampling Port. The combo-style sidearm fitting has one long tube and one short tube. The long tube on this fitting will be used to circulate medium and the short one will be fitted with a 0.22 µm pore 50 mm gas filter and will be used as a vent for the flask. Remove the sidearm fittings from their packaging, loosen the black ¼ inch nut securing the stainless steel shafts to the white plastic insert and carefully insert the shaft assemblies through the sidearms on the flask. You may have to turn and tilt the fitting in the opening and withdraw the stainless steel tubing back out of the white plastic insert in order to get the fitting into the sidearm. This sequence is shown in Figures 5, 6 and 7.
When inserted properly, the longer angled stainless steel tube on each fitting should extend down the side of the flask almost to the bottom. The shorter outlet ends of the tubes should be positioned so that approximately 2.5 to 5 cm (1 to 2 inches) protrude out of the plastic insert. Then secure the tubes by tightening the black nuts so that the tubes do not slip. **This should require finger tightening only!** Then place the orange 45 mm cap over the entire assembly and carefully screw onto the threads on the glass sidearm.

6. Attach a precut 15 cm (6 inch) piece of silicone rubber tubing to the shorter stainless steel tube on the combo-style sidearm fitting. Attach a 50 mm polytetrafluoroethylene (PTFE) gas vent filter onto the end of the tubing (Figure 8). Secure both ends of the tubing with nylon cable ties. Use of nylon cable ties to secure fitting components is shown in Figure 9. This is the **primary Venting Port** for the system.

7. Attach the longest stainless steel tube on the combo-style fitting to the open end of the silicone rubber oxygenator tubing from the top of the **Oxygenator Coil** on the E-Cube™ base. Secure with a nylon cable tie.

8. Attach a precut 15 cm (6 inch) piece of silicone rubber tubing to one of the stainless steel tubes on the dual sidearm fitting. Secure with a nylon cable tie. At the other end of the silicone tubing add a female luer fitting and secure it with a nylon cable tie. This will be used (with the addition of a septum that will be added after autoclaving) as the **Sampling Port**.

9. Attach the 61 cm (24 inch) piece of silicone rubber tubing to the remaining stainless steel tube on the dual sidearm fitting in the **Medium Reservoir**. The other end of the tubing will be attached to the left **Cell Seeding Port** in Step 11. Secure with a nylon cable tie.

**Cell Seeding Port Assembly**

10. Assemble the **Cell Seeding Ports** and the peristaltic pump tubing which will be used for the inoculating, feeding and harvesting processes of the CellCube® module. A completed **Cell Seeding Ports** diagram is shown in Figure 10.

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**Figure 8. Combo-style sidearm assembly with tubing and gas vent filter attached**

**Figure 9. Plastic nylon cable ties are used to securely attach silicone rubber tubing to all fittings.**

**Figure 10. Left and right Cell Seeding Ports diagram**
11. Starting with the left side of the assembly (Clamp A, Figure 10), thread the 61 cm (24 inches) piece of tubing from the dual side arm fitting tubing through a plastic ratchet clamp (Clamp A), positioning the clamp about 7.5 cm (3 inches) from the end of the tubing. Attach the free end of the tubing to the left barb on a T-fitting. Secure with a nylon cable tie. Attach a 15 cm (6 inch) piece of tubing on the lower barb of the T-fitting, thread a ratchet clamp (Clamp B) midway on the tubing, and attach a female luer connector to the end of the tubing. Secure all components with nylon cable ties. This is the left Cell Seeding Port.

12. Attach one end of the 45 cm (18 inch) Pharmed® Norprene® tubing (for use on a peristaltic pump) to the remaining barb on the T-fitting. Attach the other end to the left barb of the second T-fitting. Secure all components with nylon cable ties.

13. Attach a 15 cm (6 inch) piece of tubing onto the lower barb of the second T-fitting, then thread a clamp (Clamp C) midway onto the tubing and attach a female luer connector to the end of the tubing. Secure all components with nylon cable ties. This is the right Cell Seeding Port.

14. Attach a 15 cm (6 inch) length of tubing to the right barb on the second T-fitting. Thread a clamp (Clamp D) midway onto the tubing and then attach one end of the barbed straight connector to the other end of this tubing. Secure all components with nylon cable ties. The right Cell Seeding Port is now complete.

Secondary Venting Port Assembly

15. Assemble the secondary Venting Port. This port is used for venting during cell filling and harvesting procedures. A completed diagram of the secondary Venting Port is shown in Figure 11.
16. Thread the open end of the tubing from the Oxygenator Coil through a plastic ratchet clamp (Clamp F), positioning the clamp about 7.5 cm (3 inches) from the open end of the tubing. Then attach one arm of the barbed Y-connector to this tubing. Attach a precut 15 cm (6 inch) piece of silicone rubber tubing to the second arm on the Y-connector and thread on a ratchet clamp (Clamp E) positioning it midway down the tubing. Attach a female luer lock connector to the open end of this tubing. Take a 5 cm (2 inch) piece of tubing and fit a male luer lock connector to one end of the tubing and a 50 mm polytetrafluoroethylene (PTFE) gas vent filter to the other end of the tubing. Connect the tubing with the male luer lock connector and filter to the female luer lock connector on the tubing (Clamp E) from the Y-connector. Secure all connections with nylon cable ties. This is the secondary Venting Port for the system.

17. Place the Medium Reservoir into its holder. Make sure that all the tubing connections have been secured with cable ties, the tubing is not crimped and that all the tubing clamps are in the open position to allow steam to freely pass during autoclaving. Wrap the ends of the two Cell Seeding Ports, the Sampling Port and the open ends of the Y-connector and the barbed reducing connector with autoclave paper or aluminum foil. Also, sterilize some individually wrapped male luer plugs for use in sealing the female luer lock ports during use.

18. The assembled components should now resemble the diagram in Figure 12. Autoclave the system for a standard liquid cycle (121°C for 30 minutes) in an autoclave. We recommend wrapping the entire assembly prior to autoclaving with a clear autoclave bag that can act as a protective barrier during the time that the unit is cooling after autoclaving.

![Figure 12. Set up diagram for the E-Cube™ system prior to autoclaving. The CellCube® module and peristaltic pump have not yet been attached but all of the clamps are in place and in the open position. The Sampling and Cell Seeding Ports and the ends of the tubing that will connect to the CellCube module should be wrapped for autoclaving at this point.](image-url)
II. Filling the Medium Reservoir and System

19. After autoclaving, check the unit to ensure that all of the connections are still secure. Let the unit cool, preferably in a laminar flow hood and then close off all of the ratchet clamps on the system’s tubing. Remove the wrapping around the female luer connector on the dual sidearm fitting that will be used to attach the sterile septum sampler (Figure 13). Remove the wrapping around the sterile septum sampler and aseptically attach the septum to the female connector. The sterile sampling septum has a protective cap at its end that needs to be removed prior to insertion into the luer fitting. Remove the autoclave paper from the two Cell Seeding Ports and add sterile male luer plugs to seal the ports. When making sterile connections, some users like to spray the ends of the tubing or luer connectors with 70% ethanol to lower the risk of potential microbial contamination.

20. Remove the 10-stack CellCube® module from its sterile bag under the laminar flow hood, and place it in a level position so that the port marked Inlet is at the bottom facing you. This is NOT the normal operating orientation of the CellCube module, but works best during the connection to the apparatus, and subsequent filling. Carefully remove the sterile wrapping from both the CellCube inlet line and the straight connector on the Cell Seeding Ports tubing. Then aseptically connect the two lines by inserting the barb fitting on the reducing connector into the silicone rubber inlet tubing on the CellCube module.
21. Repeat for the outlet side of the CellCube® module. Carefully remove the sterile wrapping from both the CellCube outlet line and the Y-connector at the end of the Oxygenator Coil tubing. Then aseptically connect the two lines by inserting the barb fitting on the Y-connector into the silicone rubber tubing on the CellCube module. Secure all new connections, including the tubing on the CellCube module, with nylon cable ties. After the CellCube module is connected to the E-Cube™ system, place it onto its cradle. The normal operating position of the CellCube module is in a diamond pattern, with the inlet port at the bottom of the cradle facing away from the Medium Reservoir and with the outlet port facing towards the Medium Reservoir (Figure 14). Check that the inlet and outlet lines have not become crimped.

22. Aseptically fill the Medium Reservoir with approximately 1.5 L of cell culture medium. (For this step the CellCube module should be temporarily rotated in its cradle so that the outlet and inlet tubes do not interfere with opening the top of the Medium Reservoir.) This should bring the fluid level just to the level of the lowest portion of the flask sidearms. Make sure that clamps A, D and E in your flow direction are open, clamps B, C and F are closed and that all tubing connections have been secured with cable ties (Figure 15).

23. Insert the Norprene® tubing into the peristaltic pump, and slowly turn up the pump speed. Make sure that you stop pumping as soon as the CellCube module is full and before the medium reaches the Y-connector! Keep the initial medium flow rate low (approximately 100 mL/minute) while you check that the medium is flowing in the correct direction. If the medium flow is in the wrong direction, then either flip the Norprene tubing in the peristaltic pump head, or, if the pump is so equipped, reverse the flow direction. Also, check that there are no
obstructions in the line and purge any air bubbles that are present in any of the lines. If any air bubbles are found, tap the line gently to dislodge them from the sides of the silicone rubber tubing. The air in the system will be purged through the secondary Venting Port. Open clamp F and closed clamp E. Clamps A, D and F should now be open; clamps B, C and E should be closed (Figure 16). Turn on the pump again to slowly fill the remainder of the system. When the entire circulation loop is completely filled, there should be approximately 700 mL of medium remaining in the Medium Reservoir.

24. Allow the medium to circulate through the system for approximately 15 minutes to ensure that all of the connections in the fluid path are intact. Check carefully for leaks and bubbles, then turn off the pump, and move the entire assembly into a CO₂ incubator. Make sure the tubing is not crimped after you place it in the incubator. Turn on the pump and allow the system to operate undisturbed for at least two hours. This allows the gases to equilibrate through the tubing into the medium and for the medium temperature to reach the temperature (37°C for most mammalian cell lines) appropriate for the cell line.

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**Figure 16.** Flow diagram for filling the Oxygenator tubing from the Medium Reservoir. Clamps A, D and F are open; the remaining clamps are closed. The CellCube® module is already filled with medium from Step 22. This is also the flow diagram for normal system operation in Step 33.

We strongly recommend that you conduct a system check by running the system in the CO₂ incubator overnight to ensure that there are no pinhole leaks or bad connections in the system prior to introducing the cells. Also, check a sample of the medium for microbial contamination.
III. Cell Inoculation and Routine Operation

25. Prepare the inoculation bottle for seeding the CellCube® module. You will need a sterile glass or plastic 1 L inoculation bottle with a cap having two ports. One port should have a tube that reaches the bottom of the flask with a male luer lock adapter on the other end. The other port should have a hydrophobic filter membrane for venting (to prevent vacuum lock) during the process of removing the cells. An example is shown in Figure 17.

26. Prepare the cell inoculum. The 10-stack CellCube module (which has approximately 8,500 cm² of surface area) usually requires 2 to 4 x 10⁴ cells per cm² of surface area (1.7 to 3.4 x 10⁸ cells per E-Cube™ system). Some cell lines may require a higher or lower initial inoculum; this should be based on your previous experience with the cell line. Pellet the cells by centrifugation and then resuspend the cell pellet in 100 mL medium (the cell inoculum).

First Cell Seeding

27. Place 50 mL of this cell inoculum into the first inoculation bottle and place the remaining cells (for the second seeding) on ice or at 4°C. The cell inoculum for the first seeding will consist of this 50 mL of cells plus the approximately 700 mL of medium that is already in the CellCube module.

28. Turn off the pump and close all clamps. Then remove the E-Cube™ system from the incubator and place it in a laminar flow hood. When making or breaking sterile connections, it helps to spray the ends of the luer connectors with 70% ethanol to lower the risk of potential microbial contamination. Connect the inoculation bottle containing the 50 mL cell inoculum to the left Cell Seeding Port (closest to the Medium Reservoir) using the male luer lock connector. Open clamps B, D and E in that order.
Then reverse the flow direction in the peristaltic pump so that the medium in the CellCube® module will be pumped into the inoculation bottle (Figure 18, previous page). Turn on the pump until the medium from the CellCube module has drained into the bottle. Gently swirl the bottle to mix the cells and medium. Record the increase in the volume of medium now in the inoculation bottle. This volume (approximately 700 mL for a 10-stack CellCube module) represents the amount of medium required to completely fill the CellCube module. It will be used to determine the optimal amounts of washing and dissociating solutions used during cell harvesting, as well for the second cell inoculation.

29. Then reverse the pumping flow back to the original direction and turn on the pump (approximately 100 mL/minute). The medium and inoculum should now begin to flow back into the CellCube module. Gently swirl the bottle once a minute during the filling operation to prevent the cells from settling. Drain until approximately 25 mL of the inoculum remains in the bottom of the inoculation bottle. Then turn off pump and close clamps B, D and E. Remove the inoculation bottle from the left Cell Seeding Port. (Save this bottle; it will be used for the second cell inoculation in Step 31.) Add a sterile male luer plug to the female luer connector on the left Cell Seeding Port to help maintain sterility.

30. Turn the CellCube module horizontally so that the inlet line is pointing straight up and label the face of this side of the CellCube module “Side 1” using a marker so that you can distinguish it in the future. You can use the packing foam from the CellCube packaging or a small box to support the CellCube module in a horizontal position. Place it back into the incubator until the cells attach. The time required for the cells to attach can be determined based on your experience with the cell line in use, or by removing some of the cell inoculum left in the inoculation bottle and placing it into a T-25 flask. The T-flask can then be used as a model to follow the processes in the CellCube module.

If any air bubbles becomes trapped in the tubing, they should be removed by gently tapping the tubing.

Figure 19. Flow diagram for using the peristaltic pump to add the second cell inoculum into the CellCube module. Pump flow direction has been placed back into the original direction.
**Second Cell Seeding**

31. Once the cells have attached, add the remaining 50 mL of cell inoculum back into the inoculation bottle and reattach it to the left **Cell Seeding Port**. Make sure that clamps A, C and E are closed and then open clamps B, D and F in that order. Reverse the direction of the pump flow so that it flows back into the inoculation bottle when it is turned on. Run the pump until the bottle is filled to the same volume as in Step 28. Stop pumping and gently swirl the inoculation bottle to mix the cells and medium.

32. Reverse the flow of the pump back to the original direction (Figure 19, previous page) and pump until the CellCube® module has been filled with the second inoculum and all but 25 mL of medium remains. Turn off the pump and close clamps B, D and F. Remove the inoculation bottle and replace the male luer plug. Place the CellCube module onto the horizontal position on the opposite side and mark “Side 2” on the face of the CellCube module with the marker. Place the system back into the incubator for the cells to attach.

33. After the cells have attached, return the CellCube module back onto its cradle and place the entire unit in a laminar flow hood. Add fresh medium to the 1 L mark in the E-Cube™ **Medium Reservoir**. Return the system to the incubator and open clamps A, D and F and begin circulating medium at a slow speed; 50 mL/minute is recommended to start (Figure 16, page 9). This may allow slower attaching cells to potentially attach to the CellCube growth surfaces as they circulate slowly through the CellCube module.

34. We recommend slowly increasing the circulation speed through the oxygenator as the initial way of managing the process. This will help to control the pH of the medium, which typically is the first change to be observed. The length of tubing provided for oxygenation is sufficient to support about $1.0 \times 10^{11}$ cells. When cell growth has increased to a point at which pH is too low, fresh medium needs to be added. Measuring changes over time in medium metabolites, such as glucose, lactate and ammonia, can also help determine when to replace the culture medium.

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**Staying ahead of the process - a cautionary note**

As cell growth increases, events in the E-Cube™ system tend to happen at a very fast rate. The tendency for novice users is to get behind the process and then try to “play catch-up”. It is important to stress that you begin monitoring metabolic events in the CellCube module as early as possible. This allows you to alter the medium circulation rates and feeding schedule as early as possible to avoid the “feast-and-famine” situation that often occurs during the initial run in CellCube system. This also provides valuable data and training for users that will be very applicable as they move up to the larger and more complex automated CellCube systems.
IV. Sampling

35. Sampling medium from the E-Cube™ system can be done in the incubator using a blunt plastic tip cannula attached to a large syringe to pierce the sampling septum on the dual sidearm reservoir sampling port. When drawing samples, be sure to discard the first 20 mL of medium out of the Sampling Port which is not representative of the medium flowing through the system. Instead, perform analysis on a second 10 to 20 mL sample so that any of the residual material in the lines from the previous sampling is removed prior to analysis. An E-Cube system with a syringe attached to the medium reservoir sampling port is shown in Figure 20.

There are a variety of commercially available kits that can be used to test for changes in glucose, lactate and other important cell metabolites.

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Additional Required Materials

- Sterile syringes (10 to 20 mL)
- 15 mL centrifuge tubes for sample storage
V. Feeding

36. Turn off the pump, close all clamps and place the system in a laminar flow hood. Fresh medium can be introduced into the E-Cube™ system in two ways. The simplest method is to remove approximately 1/3 of the medium from the **Medium Reservoir** by aspirating or pipetting, and replace it with fresh medium (prewarmed to the temperature at which the cells are usually grown). Alternatively, an empty sterile collection bottle (Figure 17, page 10) or bag outfitted with the appropriate connectors can be connected to the **right Cell Seeding Port**. Open clamps A and C. Turn the pump back on to force the old medium into the collection bottle from the **Medium Reservoir** (Figure 21).

37. When the bottle is filled with the old medium, stop the pump and close clamps A and C. Remove the collection bottle and replace the sterile male luer plug. Then connect a bottle or bag containing replacement medium to the **left Cell Seeding Port**. Open clamps B, D and F and pump the fresh medium into the system until the bottle is nearly empty (similar to Figure 19, page 11). Try to avoid introducing air into the tubing in the E-Cube system. Turn off the pump and close all clamps. Then remove the empty bottle or bag and replace the sterile male luer plug. Return the system to the incubator and open clamps A, D and F and turn on the pump to resume normal operation (Figure 16, page 9).

**If any air bubbles becomes trapped in the tubing, they should be removed by gently tapping the tubing.**

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**Additional Required Materials**
- 500 mL to 1.5 L culture medium in bottle or bag with appropriate ports and connectors
- 1 to 2 L empty bottle or bag with appropriate ports and connectors
VI. Harvesting

38. Turn off the pump and close all of the clamps. Move the E-Cube™ system to a laminar flow hood. Connect an empty sterile 2 L waste bottle or bag with the appropriate connectors to the right Cell Seeding Port. Open clamps A and C, turn on the pump and run it at twice the normal speed. The medium will be pumped from the Medium Reservoir into the waste bottle or bag (Figure 21). When the Medium Reservoir is empty, turn the pump off and close clamp A. Open clamps D and F, and allow the CellCube® module to empty into the waste collection bottle by gravity. Lowering the waste collection bottle will speed up the draining process. When the CellCube module is empty close all clamps and remove the waste collection bottle. Replace the sterile male luer plug.

39. To wash the cells in the empty CellCube module, connect a bottle or bag containing 2 L wash solution to the left Cell Seeding Port. Open clamps B, D and F in that order. Turn on the pump so that the wash solution goes through the CellCube module into the Medium Reservoir (Figure 22).

40. Wash the CellCube module with at least three times its volume (approximately 1800 mL) until the bottle is empty. Then turn the pump off and close clamp F and open clamp E. Reverse the pump flow direction and pump the CellCube module’s contents back into the wash solution bottle until the CellCube module is empty. Turn pump off and close all of the clamps.

41. Replace the wash buffer bottle on the left Cell Seeding Port with a bottle containing approximately 700 mL dissociating solution (same volume as used in Step 28) prewarmed to the temperature at which the cells are grown. Remove the vent filter and tubing by disconnecting the male luer adapter from the female luer. Connect a 2 L harvesting bottle to the

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**Additional Required Materials**

- 700 mL culture medium in 1 L bottle or bag with appropriate ports and connectors
- 2 L wash solution in 2 L bottle or bag with appropriate ports and connectors
- 2 L harvesting bottle or bag with appropriate ports and connectors
- 700 mL dissociating solution in 1 L bottle or bag with appropriate ports and connectors

We recommend using calcium- and magnesium-free phosphate buffered saline as the wash solution. The same buffered solution can be used with the dissociating reagents (mixture of trypsin and EDTA or other proteolytic enzymes).
**secondary Venting Port.** Open clamps B, D and E (Figure 23) and reverse the pump flow direction. Turn on the pump to fill the CellCube™ module with the dissociating solution and pump until the dissociating solution reaches the Y-connector near the harvesting bottle.

42. Immediately switch the pump flow direction back and forth at a very low speed so that the dissociating solution flows back and forth across the cell layers inside the CellCube module to dislodge the cells more quickly. Hitting the CellCube module on the narrow (white) side with your hand will also detach cells more quickly. Keeping the dissociating solution moving slowly back and forth through the CellCube module also reduces cell clumping.

43. Once the cells have begun to detach, place the CellCube module upside down onto its cradle. The outlet of the CellCube module is now on the bottom. Fill (or replace) the empty dissociating solution bottle with 700 mL of culture medium. Pump until all of the medium is in the harvesting bottle and the CellCube module is empty. Turn the pump off and close all of the clamps. The harvesting solution should now contain all of the cells in a mixture of dissociating solution and culture medium.

44. Transfer the harvested cell solution to 250 or 500 mL centrifuge tubes or bottles and spin at 100xg for 5 to 10 minutes to pellet the cells. Remove and discard the supernatant and resuspend cells in medium. Count the cells to determine yield.
References

Retroviral vector production in a Corning® CellCube® system was compared with production in roller bottles. The CellCube system gave significantly higher (4 to 5 times) titers than in roller bottles, was easy to use and scale up.

The CellCube system was used to successfully propagate recombinant CHO (Chinese hamster ovary expressing the NK2 receptor), BHK (Baby hamster kidney), COS M6 (Green monkey kidney) and SKMNC (Human neuroblastoma) cell lines.

High titer retroviral vector production in human-based packaging cell line (TE Fly GA 18) was compared in a Corning CellCube system, in a New Brunswick Celligen™ Cellift stirred tank system (both on microcarriers and clump cultures) and New Brunswick Fibra-Cel™ Basket reactor. Viral titers and cell growth were highest in the CellCube system and were clearly superior to the stirred tank system.

Production of a Herpes Simplex virus was optimized in a CellCube system that gave virus yields in 7b cells better than in flasks or roller bottles with significant savings in time, labor and costs.

The CellCube system was compared with Nunc™ Cell Factories, microcarriers and static mixer reactors for the production of Hepatitis A virus in MRC-5 cells for use in a vaccine. The authors chose the CellCube system for commercial vaccine production based on its technical success, ease of use, consistency, ability to meet regulatory demands, and low manufacturing costs.
Corning® E-Cube System Ordering Information

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**Replacement parts**

3287 E-Cube Replacement Parts Kit (Contains plastic fittings and clamps for the E-Cube System. Does not contain the Medium Reservoir, Base, silicone rubber tubing or CellCube module) 1

4500-1L 1 L Spinner Flask® (Complete) 1

*Additional information on Corning ProCulture™ Spinner Flask Accessories can be found under Product Literature, Laboratory Glassware on the Corning Life Sciences web site.

**Accessories**

1395-1L I L Borosilicate Glass Storage Bottle with 45 mm Cap NA 10

430518 Sterile 1 L Polystyrene Storage Bottle with 45 mm Cap 2 24

401654 45 mm Cap with 2 Stainless Steel Tubes 1 1

430776 250 mL Sterile Polypropylene Centrifuge Tubes 6 102

431123 500 mL Sterile Polypropylene Centrifuge Tubes 6 36

431227 0.2 µm pore Gas Vent Filter (50 mm Diameter) 1 12

For additional product or technical information, please visit our web site at www.corning.com/lifesciences or call 1-800-492-1110. Outside the United States call 978-635-2200.