Corning® CellSTACK® Culture Chambers

Instructions for Use

Introduction

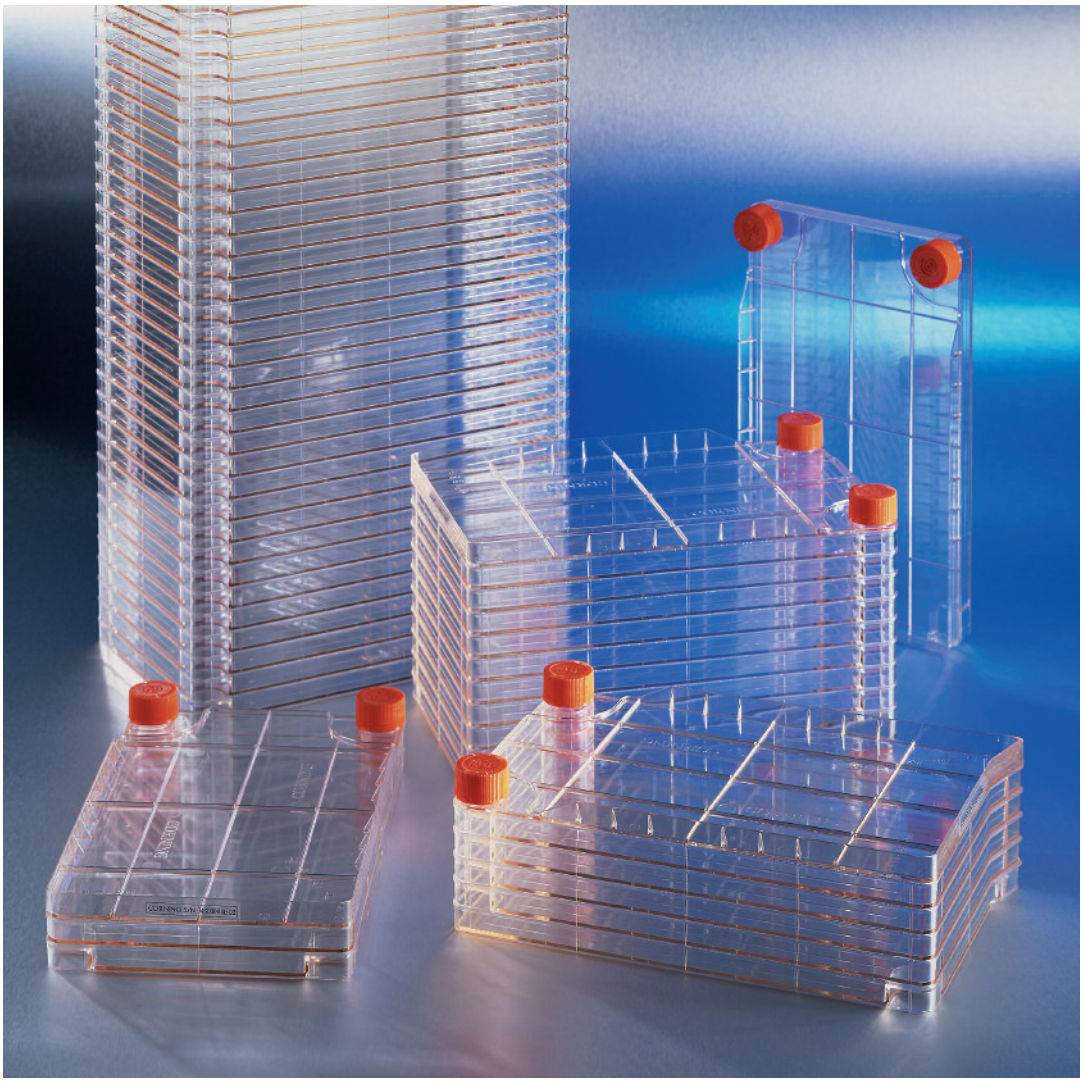
CellSTACK Culture Chambers are one of Corning Life Sciences' most reliable and fully tested cell culture products.

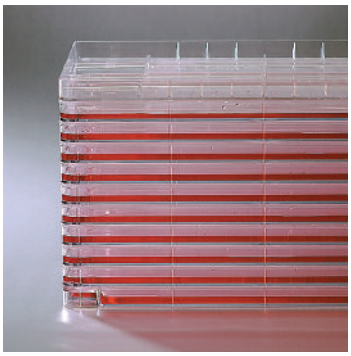
Available in five sizes:

- 1-STACK with 636 cm^2 cell growth area
- 2-STACK with 1,272 cm^2 cell growth area
- 5-STACK with 3,180 cm^2 cell growth area
- 10-STACK with 6,360 cm^2 cell growth area
- 40-STACK with 25,440 cm^2 cell growth area

Two 26 mm diameter filling and venting ports allow direct access to the chamber bottom providing greater flexibility for sterile filling and emptying by pouring, pipetting or via tubing in a fully closed system. The standard 33 mm threaded caps have 0.2 µm pore, hydrophobic membranes sealed directly to the caps, allowing gas exchange while minimizing the risk of contamination.

Optional filling caps are available with integrally sealed USP Class VI certified C-FLEX® tubing to allow direct sterile transfer of media and cells via pumping or gravity.





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The following steps are designed for filling the chamber by gravity but can be easily adapted to work with a peristaltic pump (Step 8) or by pouring (Step 9).

1

Unpack the CellSTACK Chambers. All aseptic connections should be made in a laminar flow hood or clean room environment. Once they are done, chambers can be moved to a nonsterile environment if a closed filling system (via gravity or pumping) is used.

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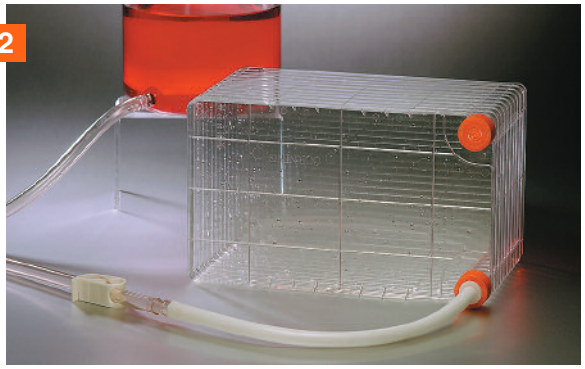

Replace the standard vent cap on the filling port with a filling cap (Corning Cat. No. 3282 or 3283) and place the chamber on its side with the filling port near the bottom. **For filling with fast flow rates (up to 2L/minute), the remaining standard vented cap on the venting port must be replaced with a vented filling cap with 50 mm air venting filter (Corning Cat. No. 3281, shown in Step 8) to reduce back pressure.** Failure to do this will result in a back pressure build up that can crack the chamber. Connect the filling cap tubing to the tubing (a clamp should be in place) from a sterile dispensing vessel containing the cell suspension.

Recommended CellSTACK medium volumes (mL)
1-STACK: 130 to 200
2-STACK: 260 to 400
5-STACK: 650 to 1,000
10-STACK: 1,300 to 2,000
40-STACK: 5,200 to 8,000

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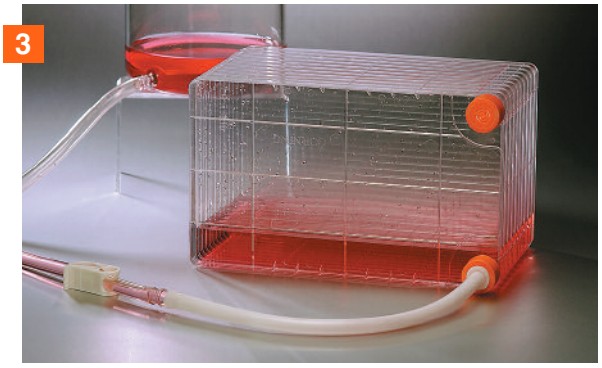

Elevate the dispensing vessel containing the cell suspension above the CellSTACK Chamber. Loosen the clamp to allow the cell suspension to flow into the chamber. The chambers may initially fill unevenly but the medium will quickly level out in each chamber. If pressure develops inside the chamber during filling, then (1) briefly loosen the vented cap with filter to allow the pressure to vent faster, (2) slow down the filling rate, or (3) use a different filling technique.

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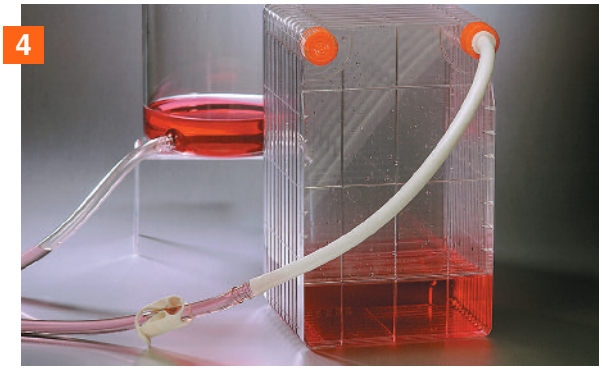

Once filling is complete, turn the CellSTACK Chamber 90° so that the filling and venting ports are up (as shown). It is normal for the medium level in the bottom chamber section to be slightly higher. Replace the cap on the filling port with the original vented cap, a vented cap with 50 mm filter (Corning Cat. No. 3281), or a solid cap (Corning Cat. No. 3969).

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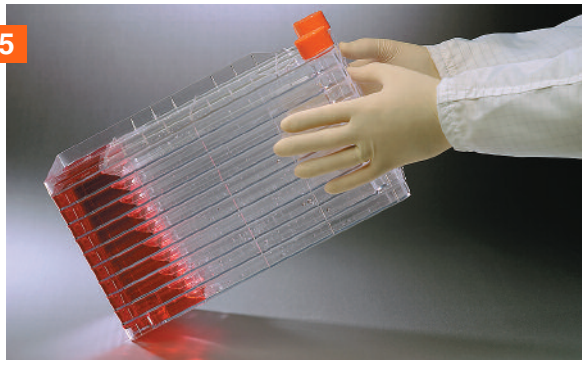

Gently lower the CellSTACK Chamber to its normal horizontal incubation position and gently tilt the chamber back and forth until the surface of each chamber is completely covered with medium. This will ensure an even distribution of cells across each of the growth surfaces. **Be careful when tilting to not allow medium to flow over the edge of the chambers into the access column as this will result in more cells and medium in the lower chamber levels. This problem can be corrected by repeating Steps 3, 4, and 5.**

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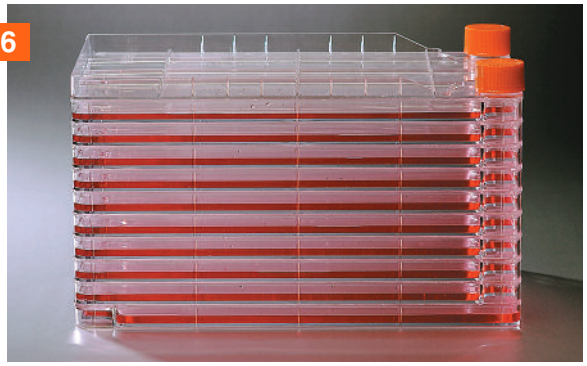

If more gas exchange is necessary, replace one or both of the vented caps with a cap containing a 50 mm vent filter with 0.2 µm pore hydrophobic membrane (Corning Cat. No. 3281; see picture in Step 8). Place the CellSTACK® Culture Chamber into an incubator or warm room. **It is very important to ensure the incubator shelves are level and can fully support the weight of CellSTACK Chambers.**

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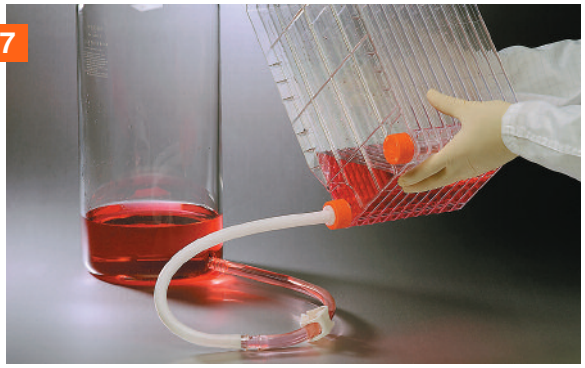

To empty a CellSTACK Chamber, repeat step 2 by replacing the cap on the filling port with a filling cap. Place the chamber above a sterile collection vessel and transfer the medium by gravity or by pumping (step 8). **Do not loosen the vented cap in a nonsterile environment when emptying chamber.** This can pull in contamination due to a negative back pressure. For information on cell harvesting, see Helpful Hint 5.

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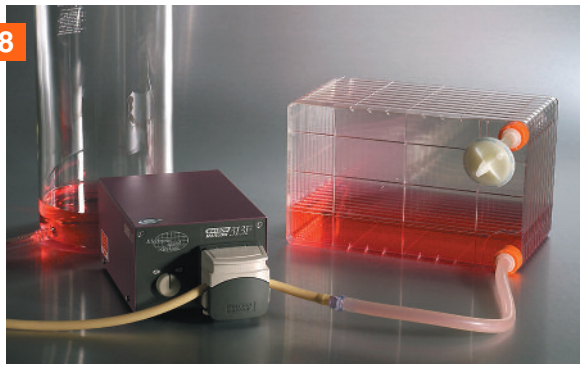

CellSTACK Chambers can also be filled and emptied using peristaltic pumps. The faster flow rates obtained by pumping can increase cell damage from shearing and media foaming. Back pressures can also be created that can crack the chambers. **To prevent this, the standard venting cap on the venting port must be replaced with a cap with a 50 mm air venting filter (Corning Cat. No. 3281) and pumping flow rates must not exceed 2L/minute. For flow rates greater than 2L/minute, a higher capacity air venting filter (Corning Cat. No. 3284) is required.**

9

CellSTACK Chambers can also be easily filled by carefully pouring the medium from a sterile bottle or using a filling bell (Step 10). Tilting the chamber slightly towards the filling port while filling will reduce foaming. The chamber will then have to be placed on its side (Step 3) and the procedure for equilibrating the medium in each chamber section performed (Step 4). Smaller CellSTACK Chambers can also be easily emptied by carefully pouring from the filling port into a sterile collection vessel.

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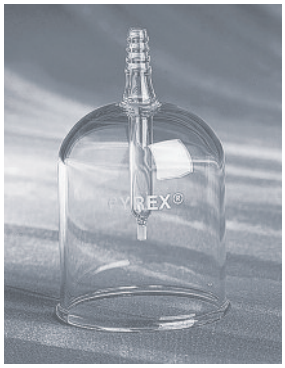

PYREX® aspirator bottles (above left, Corning Cat. No. 1220, available in 250 mL to 19L capacities) fitted with silicone rubber stoppers and autoclavable 50 mm vent filters (Corning Cat. No. 431227) are recommended for aseptically dispensing and collecting media from CellSTACK Chambers. PYREX filling bells (above right, Corning Cat. No. 3960-L) can be used in conjunction with the aspirator bottles to provide more secure aseptic filling.

CellSTACK Chambers are also available with Corning® CellBIND™ Surface for enhanced cell attachment.

	Standard Tissue Culture Treatment Cat. No.	Corning CellBIND Surface Cat. No.	Qty/Cs
1-STACK	3268	3330	8
2-STACK	3269	3310	5
5-STACK	3319	3311	2
10-STACK	3270	3312	2
10-STACK	3271	3320	6
40-STACK	3272	3321	2

Corning® CellSTACK® Culture Chambers – Helpful Hints

1. Cell seeding densities and media volumes should be proportional to those used in standard cell culture flasks and dishes. A 10-STACK Chamber has approximately 85 times the growth surface area of a 75 cm^2 flask and thus would require 85 times the cell seeding density and medium volume used for the flask. Corning recommends using between 130 and 200 mL (0.2 to 0.3 mL/cm^2) cell culture medium for each chamber.
2. Continued mixing of the cell suspension (gently as to not create foam) when filling chambers is recommended to ensure even cell distribution. Preheating the medium to incubation temperature is also recommended to ensure rapid cell attachment. **For larger CellSTACK Chambers, prewarming the Chamber and the medium is especially important due to the increased time required for the middle chambers to reach desired incubation temperatures.**
3. Cell growth in 1-STACK and 2-STACK Chambers can be monitored on standard inverted microscopes. Growth in the larger CellSTACK Chambers can be monitored indirectly by setting up a companion culture in a 1-STACK Chamber or a Corning® T-flask with proportional cell density and medium volume, or by measuring other parameters such as glucose utilization or lactic acid production.
4. Be careful not to spray the hydrophobic filters on the venting caps with alcohol as this will wet them and potentially block the gas flow resulting in pressure build-up or preventing gas exchange.
5. For enzymatic cell harvesting using trypsin or other dissociating enzymes, Corning recommends washing the chamber with calcium- and magnesium-free phosphate buffered saline (40 to 50 mL per chamber) at least once to remove any serum-containing medium residues. Then add prewarmed dissociating solution (20 to 30 mL per chamber) to the chamber and carefully tilt the chamber back and forth and side to side to ensure the entire cell surface is completely wetted with dissociating solution. For more detailed information check the CellSTACK Chamber Cell Harvesting protocol on the Corning web site at **www.corning.com/lifesciences**.
6. Check temperature of shelves as well as the air at different levels in walk-in warm rooms. A 1°C to 2°C variation from the required optimal cell growing temperature is common in many warm rooms and may decrease desired yield significantly.

Corning CellSTACK Filling Accessories Ordering Information

Cat. No.	Description	Qty/Pk	Pk/Cs
3969	Solid Cap, 33 mm threaded cap	1	6
3968	Vented Cap, 33 mm threaded cap with 0.2 µm pore hydrophobic membrane	1	6
3281	Vented Filling Cap, 33 mm threaded cap with 3/8" (9.5 mm) ID tubing and 50 mm filter with 0.2 µm pore hydrophobic membrane	1	5
3282	Filling Cap, 33 mm threaded cap with 1/8" (3.2 mm) ID tubing and a female Luer 1/8" (3.2 mm) hose barb with male Luer lock plug	1	5
3283	Filling Cap, 33 mm threaded cap with 3/8" (9.5 mm) ID tubing and 5/16" (7.94 mm) ID barbed fitting	1	5
3284	Vented Filling Cap, 33 mm threaded cap with 3/8" (9.5 mm) ID tubing and 37 mm filter with glass fiber hydrophic membrane	1	4
3332	Universal Cap, 33 mm threaded cap with .468" (12 mm) opening, vented cover cap and pouched solid cover cap	1	4
3333	Fill cap, 1/4" (6.4 mm) ID tubing, 70 cm length, male MPC coupling with female end cap	1	4

Customized accessories are available upon request. For additional product or technical information, please e-mail us at **clstechserv@corning.com**, visit **www.corning.com/lifesciences**, or call 800.492.1110. Customers outside the United States, call +1.978.442.2200 or contact your local Corning sales office listed below.

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