Corning[®] CellSTACK[®] Culture Chambers

Instructions for Use

CORNING

Introduction

Corning CellSTACK Culture Chambers are stacked unit vessels designed to meet the need of both suspension and adherent cellculture applications. Scientists in research laboratories and industrial processing facilities around the world are using them to scale up cell-based production.

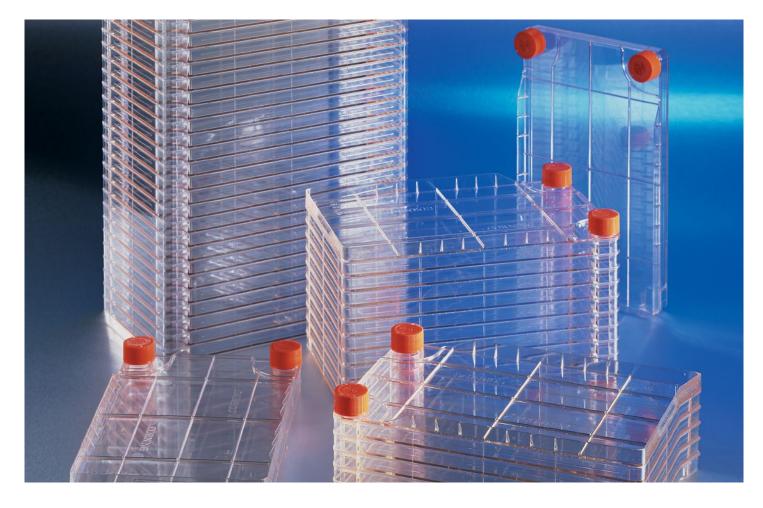
CellSTACK vessels are available with traditional tissue culture surface, Corning CellBIND® surface for enhanced cell attachment, and Ultra-Low Attachment surface for decreased cell attachment (1-stack version only). Each CellSTACK vessel layer offers 636 cm² of cell growth area. They are available in five sizes:

- 1-Stack with 636 cm² cell growth area
- > 2-Stack with 1,272 cm² cell growth area
- ▶ 5-Stack with 3,180 cm² cell growth area
- ▶ 10-Stack with 6,360 cm² cell growth area
- 40-Stack with 25,440 cm² cell growth area

The top of the vessel is equipped with two 26 mm diameter filling and venting ports, which provide direct access to the chamber bottom and allow for greater flexibility during sterile manipulations. The standard 33 mm threaded caps feature 0.2 micron pore size and hydrophobic membranes sealed directly to the caps, allowing gas exchange.

Filling caps are available with integrally sealed USP Class VI chemically resistant, heat sealable flexible tubing to allow direct transfer of media and cells via pumping or gravity.

In addition, Corning offers an extensive library of validated components including filters, connectors, tubing, clamps, and plugs, making it easy to design a customized closed system solution for your specific application. Please contact your local Corning Representative for more information.



The following protocol requires a Corning[®] CellSTACK[®] filling cap (Cat. Nos. 3282, 3283, 11902, or 3333) and may require an air venting filter (Cat. No. 3281).



Unpack the CellSTACK vessel, and place it inside a laminar flow hood or clean room environment to carry out aseptic connections.



Recommended Corning 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

Replace one vent cap with a filling cap. Place the CellSTACK vessel on its side with the filling cap toward the bottom of the vessel. The standard vent cap allows for gravity filling.

CAUTION: Fill rates greater than 1.2 L/min. can result in overpressurization of the vessel and lead to cracking.



Ensure the dispensing vessel is elevated above the CellSTACK vessel. Connect the MPC from the filling cap to the MPC from the filling vessel. Open the clamp to start filling. Medium will level out between layers during filling.



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 filling cap with the original vented cap and the vented cap with a venting filter or solid cap (Cat. No. 3969).



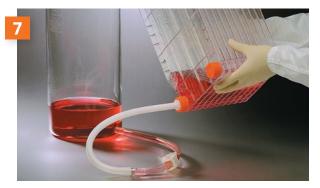
Gently lower the CellSTACK chamber to its normal horizontal incubation position, and gently tilt the chamber back and forth until the surface of each chamber is completely covered with medium. This will ensure an even distribution of cells across each of the growth surfaces. **NOTE:** Be careful when 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.



For additional gas exchange during incubation, one or both venting caps can be replaced with a higher gas flow filter. Place the CellSTACK vessel into an incubator or warm room.

CAUTION: Ensure incubator shelves are level and able to withstand the weight of the CellSTACK vessel.

CAUTION: Due to pressure build-up during incubation, CellSTACK vessels must be vented during incubation.



To empty the CellSTACK vessel, replace one of the vented caps with a filling cap as in Step 2. Ensure the CellSTACK vessel is above the sterile collection vessel if emptying by gravity.

Alternate Filling, Emptying Methods



If using a peristaltic pump for filling and emptying, it is recommended to replace the vent cap with a venting filter to prevent potential shearing, media foaming, or cracking of the vessel.

CAUTION: Fill rates greater than 1.2 L/min. can result in overpressurization of the vessel and lead to cracking.



To fill the CellSTACK vessel by hand, tilt the vessel slightly toward the filling port. Smaller CellSTACK vessels can also be emptied by hand by carefully pouring from the filling port into a sterile collection vessel.

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 cm² 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/cm²) cell culture medium for each chamber.
- 2. Continued mixing of the cell suspension (gently as to not create foam) when filling the chambers is recommended to ensure even cell distribution. Preheating the medium to incubation temperature is also recommended to ensure rapid cell attachment.

NOTE: 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. The vented caps contain a hydrophobic filter which will repel any media inside the CellSTACK when equilibrating.

CAUTION: Be sure the vented caps do not come into contact with alcohol, as this could block the gas exchange, resulting in pressure build-up or inadequate 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 Corning CellSTACK Chamber Cell Harvesting protocol (Lit. Code. CLS-AN-076) at www.corning.com/lifesciences.
- 6. Check the 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.

Ordering Information

Corning[®] CellSTACK[®] Culture Chambers

| Cat. No. | Description | Surface | Growth Area (cm ²) | Qty/Pk | Pk/Cs |
|----------|----------------------|----------------------|--------------------------------|--------|-------|
| 3303 | CellSTACK 1-chamber | Ultra-Low Attachment | 636 | 1 | 8 |
| 3330 | CellSTACK 1-chamber | Corning CellBIND® | 636 | 1 | 8 |
| 3268 | CellSTACK 1-chamber | TC-treated | 636 | 1 | 8 |
| 3310 | CellSTACK 2-chamber | Corning CellBIND | 1,272 | 1 | 5 |
| 3269 | CellSTACK 2-chamber | TC-treated | 1,272 | 1 | 5 |
| 3311 | CellSTACK 5-chamber | Corning CellBIND | 3,180 | 1 | 2 |
| 3319 | CellSTACK 5-chamber | TC-treated | 3,180 | 1 | 2 |
| 3313 | CellSTACK 5-chamber | TC-treated | 3,180 | 1 | 8 |
| 3320 | CellSTACK 10-chamber | Corning CellBIND | 6,360 | 1 | 6 |
| 3312 | CellSTACK 10-chamber | Corning CellBIND | 6,360 | 1 | 2 |
| 3270 | CellSTACK 10-chamber | TC-treated | 6,360 | 1 | 2 |
| 3271 | CellSTACK 10-chamber | TC-treated | 6,360 | 1 | 6 |
| 3321 | CellSTACK 40-chamber | Corning CellBIND | 25,440 | 1 | 2 |
| 3272 | CellSTACK 40-chamber | TC-treated | 25,440 | 1 | 2 |

Corning CellSTACK Filling Accessories

| Cat. No. | Description | Qty/Pk | Pk/Cs |
|----------|---|--------|-------|
| 3969 | Solid cap, 33 mm threaded cap | | 6 |
| 3968 | Vented cap, 33 mm threaded cap with 0.2 μ m 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 ½" (3.2 mm) ID tubing and a female Luer ½" (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 |
| 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 more specific information on claims, visit www.corning.com/certificates.

Warranty/Disclaimer: Unless otherwise specified, these products are US class I medical devices for cell and tissue culture. These products are not intended to mitigate the presence of microorganisms on surfaces or in the environment, where such organisms can be deleterious to humans or the environment. Corning does not make or support any further claim for these products nor does it conduct any further testing to support any other intended use for the product indicated above. Should you decide that you want to use the product for any intended use other than that made by Corning then you accept that Corning does not make or support that claim and that you as the user are responsible for any testing, validation, and/or regulatory submissions that may be required to support the safety and efficacy of your intended application.

CORNING

Corning Incorporated Life Sciences

www.corning.com/lifesciences

NORTH AMERICA t 800.492.1110 t 978.442.2200

ASIA/PACIFIC Australia/New Zealand t 61 427286832 Chinese Mainland t 86 21 3338 4338

India t 91 124 4604000 Japan t 81 3-3586 1996 Korea t 82 2-796-9500 Singapore t 65 6572-9740 Taiwan t 886 2-2716-0338 EUROPE CSEurope@corning.com France t 0800 916 882 Germany t 0800 101 1153 The Netherlands t 020 655 79 28 United Kingdom t 0800 376 8660 All Other European Countries t+31 (0) 206 59 60 51

LATIN AMERICA

grupoLA@corning.com Brazil t 55 (11) 3089-7400 Mexico t (52-81) 8158-8400