

# Roller Bottle Aseptic Transfer Cap User Guide

## Protocol

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### Introduction

The Roller Bottle **Aseptic Transfer Cap (ATC)** accessory was designed to facilitate the closed system liquid handling of the Corning® *HYPERStack*® 12-Layer Cell Culture Vessel and *CellSTACK*® Culture Chambers, (2 to 10 layer formats). Fluid movement is facilitated by positive or negative pressure using a vacuum pump to pressurize (fill) or apply a vacuum (empty) to the cell culture vessel.

### Materials

- ▶ Corning Roller Bottle Aseptic Transfer Cap (Cat. No. 10043)
- ▶ Corning 850 cm<sup>2</sup> roller bottles, sterile, not treated (Cat. No. 431644)
- ▶ Corning 33 mm filling cap (3/8" ID tubing) with a female medical plastic coupler (MPC) connector with cap (Cat. No. 3329)\*
- ▶ Corning 33 mm filter cap (3/8" ID tubing) with 0.2 µm filter (Cat. No. 3281)\*\*
- ▶ Low vacuum/pressure pump

### Method

#### Setting up the Liquid Transfer Cap

1. Using aseptic techniques and working under sterile conditions, prepare cell suspension to desired concentration in the sterile roller bottle (maximum fill volume is 2L, use not treated roller bottle).
  - *HYPERStack*-12 fill volume = 1.3L (0.217 mL/cm<sup>2</sup>)
  - *CellSTACK* Culture Chamber, recommended volume = 0.2 to 0.3 mL/cm<sup>2</sup>
2. Aseptically, remove the roller bottle standard cap and replace with the ATC. Avoid contaminating the ATC's dip tube.
3. Tighten the ATC onto the roller bottle.
4. Adjust vacuum/pressure pump to pressure setting, set regulator to 1.5 pounds per square inch (psi) (~10.3 kilopascals [kPa]).
5. Connect the pump tubing to the 0.2 µm vent filter on the ATC.

#### Connecting to Vessel

- ▶ *HYPERStack*-12 vessel:
  - Using aseptic techniques and working under sterile conditions, connect the MPC on the ATC to the MPC on the *HYPERStack* vessel.
- ▶ *CellSTACK* culture chambers:
  - Replace one of the vessel's caps with a filling cap with MPC connector then connect to ATC.
  - If working with 5 or 10 layer vessels, replace second cap with the filter cap.

**NOTE:** Alternatively, the ATC tubing can be welded to the tubing of the receiving vessel.

\*Necessary if working with *CellSTACK* Culture Chambers.

\*\*Recommended if working with 5 and 10 layer *CellSTACK* Culture Chambers.



Roller Bottle Aseptic Transfer  
Cap

## Filling a Vessel

*Follow user instructions of corresponding vessel for correct orientation/positioning of vessel during filling.*

6. Open the liquid-handling tubing clamps on the ATC and the receiving vessel tubing.
7. Turn on the pump to pressurize the roller bottle and push the fluid up the dip tube of the ATC and into the connected vessel.
  - If rate of fill is too slow, increase pressure making sure to not exceed 1.5 psi (10.34 kPa).
8. Turn the pump off when approximately 15 mL of fluid remains to be transferred. The residual pressure in the roller bottle will force the remaining fluid into the receiving vessel.
9. Close the liquid-handling tubing clamps once the appropriate volume has transferred.
10. Disconnect the pump tubing from the ATC vent filter.
11. Completely empty all liquid from tubing, then disconnect the ATC from the receiving vessel.
12. Recap MPC connectors to maintain sterility. Liquid transfer is complete.
  - CellSTACK® vessel fill and vent caps do not need to be replaced; they can remain on the vessel during incubation.
13. Equilibrate and distribute liquid in the vessel as recommended in user manual.

## Emptying a Vessel

*Follow user instructions of corresponding vessel for correct orientation/positioning of vessel during emptying.*

14. Using aseptic techniques and working under sterile conditions, replace the roller bottle's standard cap with the ATC.
  15. Tighten the ATC onto the roller bottle.
  16. Adjust vacuum/pressure pump to vacuum setting.
    - Recommended vacuum setting: 15 inHg (380 mmHg, 50 kPa)
  17. Connect the pump tubing to the 0.2 µm vent filter on the ATC.
  18. Using the MPC connectors, connect the ATC to the vessel.
- NOTE:** Alternatively, the ATC tubing can be welded to the tubing of the receiving vessel.
19. Open the liquid-handling tubing clamps on the ATC and the tubing to the vessel being emptied.
  20. Turn the pump on to create vacuum in the roller bottle to pull the fluid from the vessel to be emptied into the roller bottle.
    - If rate of emptying is too slow, increase vacuum, making sure to not exceed 20 inHg (508 mmHg, 68 kPa).
  21. Turn off the pump once the appropriate volume of fluid is transferred into the roller bottle.
    - Be careful not to draw fluid into the vent filter tubing or wet the filter.
  22. Close the liquid-handling tubing clamps.
    - Disconnect the pump tubing from the ATC vent filter.
  23. Air from the atmosphere may be drawn into the roller bottle after vacuum is broken. The ATC vent filter will prevent contamination.
  24. Completely empty all liquid from tubing, then disconnect the ATC from the vessel.
  25. Re-cap the MPC connectors to maintain sterility. Liquid transfer is complete.

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