Corning® 2L Gas-permeable Cell Culture Bag

Handling Guidelines

CORNING

Introduction

Corning® gas-permeable cell culture bags can be used to expand and culture non-adherent cells, such as T cells, NK cells, and human lymphocytes. The containers are made from USP Class VI ethyl vinyl acetate (EVA) film, the bag material is gas-permeable and transparent for use in microscopy applications, and the integrated tubing allows for functionally closed system filling, inoculation, incubation, sampling, and harvesting. Corning® gas-permeable cell culture bags are a closed system, greatly reducing the possibility of contamination. All bags are sterile and have one female Luer lock and one septum port; this septum port is reusable to allow for sampling (Figure 1). This single-use cell culture bag is ideal for basic research biopharmaceutical applications.

Features and Benefits

- Cell expansion observed with multiple cell models
- Gas-permeable film
- Reusable sampling septum port for process testing
- Tubing for sterile weld connections

Materials

- Corning gas-permeable cell culture bag (Corning Cat. No. 88-610-20)
- Corning 500 mL centrifuge tube with male Luer lock dip tube (Corning Cat. No.11750)
- Syringe or syringe with a male Luer lock connection fitting
- Sterile docking device

Instructions for Use

These instructions provide practical guidelines for filling, inoculation, incubation, and harvesting of cells using Corning® gas-permeable cell culture bags. The procedure may vary depending on the customer's specific application.

1. Preparing cell suspension medium

Prepare a sufficient amount of medium according to your laboratory protocol. Large volumes (100–2,000 mL) of medium can be aseptically transferred into Corning gas-permeable cell culture bags using a sterile device.

Note: For cell culture, do not exceed the maximum fill volume. It is recommended that the thickness of the media does not exceed 1 cm when the bag is flat.

2. Inoculation and replenishment

- Place one cell culture bag and sterile docking device (Figure 2) in a biosafety hood.
- Using a 50 mL syringe, remove the syringe plug. Insert the syringe sleeve into the round hole of the docking device. For this step, a 50 mL syringe with a Luer-lock tip (BD Cat. No. 309653) is recommended.
- Remove the male Luer cap of the cell culture bag tube and connect the female Luer tube with the syringe (Figure 3). Place the cell culture bag horizontally on the hood.
- Add the cell suspension medium by pouring or pipetting (Figure 4). Open the clamp and

allow the culture medium to drain into the cell culture bag until the required volume is achieved, as indicated on a balance.

- Holding the cell culture bag tube in an upright position, remove as much excess air from the bag as possible until the medium reaches the tubing (Figure 5).
- Clamp the tubing as close to the bag as possible.
- Remove the syringe and screw the male Luer cap onto the female Luer lock on the tubing.
- Add fresh medium using the above procedure.

3. Incubation

- Place the cell culture bags horizontally on an incubator shelf (Figure 6). Ensure that the incubator shelf is level and the cell culture bag is completely flat to allow for optimal cell expansion. The integral tubing set can be placed next to or on top of each cell culture bag.

Note: Never stack or overlap cell culture bags in the incubator.

- o allow for optimal gas exchange, the surface of the cell culture bag must remain unobstructed.
- To view the contents of the cell culture bag, place the bag directly on a microscope stage, as the EVA film is optically clear and can be observed directly.

4. Cell culture sampling

- Insert the needle of the syringe into the septum port of the cell culture bag.
- Gently rock the cell bag to suspend the cells. Fill and empty the syringe several times with the cell suspension by pulling and pushing the plunger of the syringe (Figure 7). This ensures the removal of a statistically valid sample.
- Remove the required sample.

Note: Disinfect the septum port before and after use with 70% alcohol.

5. Cell culture harvest

- Gently mix the contents of the cell culture bag to resuspend the cells. Cells can be suspended by rocking the culture bag and forth with both hands approximately 20–30 times.
- Connect the tubing of the Corning 500 mL centrifuge tube to the tubing of the cell culture bag using the male and female ports.
- Invert the cell culture bag and open the clamp.
- Transfer the cell culture suspension by gravity from the cell culture bag to a centrifuge tube.
- Keep replacing the centrifuge tubes during cell suspension transfer until the entire culture volume has been harvested. The cell culture bag can be rinsed by injecting additional wash solution and transferring the solution to a centrifuge tube.
- Pellet the suspension in the centrifuge tube by centrifugation according to your institution's protocol (e.g., 800 x g for 10 minutes).
- If cell washing is required, the centrifuge tube containing the cell pellet can be connected to the tubing of a bag containing wash buffer. Transfer the wash buffer to the cell pellet, resuspend the cell pellet, and centrifuge.
- The cells are now ready for final processing according to your institution's protocol.

Figure 1. Corning gas-permeable cell culture bags are equipped with a septum port (up) and tubing with a female Luer lock and cap (down).

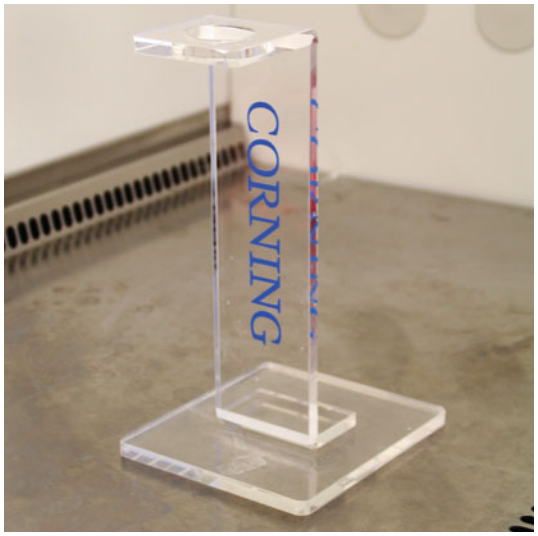


Figure 2. Corning cell culture bag sterile docking device.

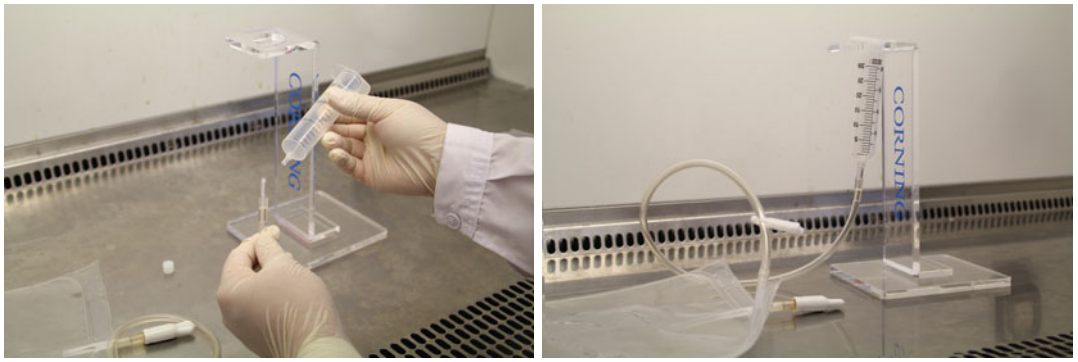


Figure 3. Syringe attached to the female Luer lock of the Corning cell culture bag.

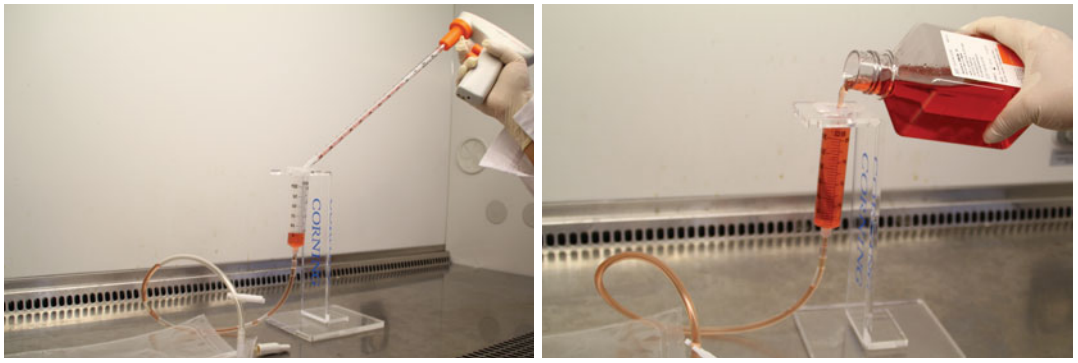
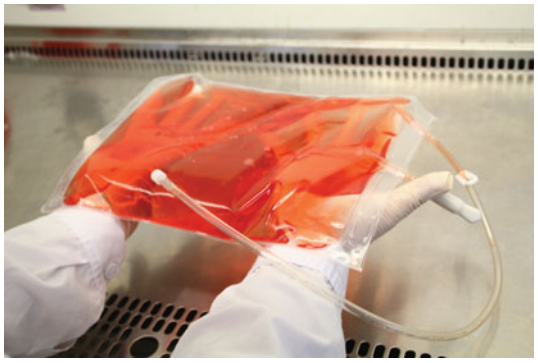


Figure 4. Filling the Corning cell culture bag by pipetting (left) and pouring (right).

Figure 5. Remove as much excess air from the Corning cell culture bag as possible.



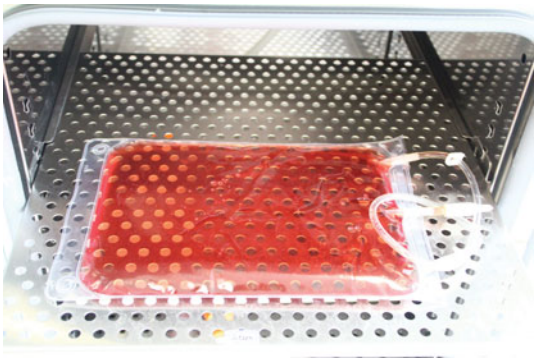
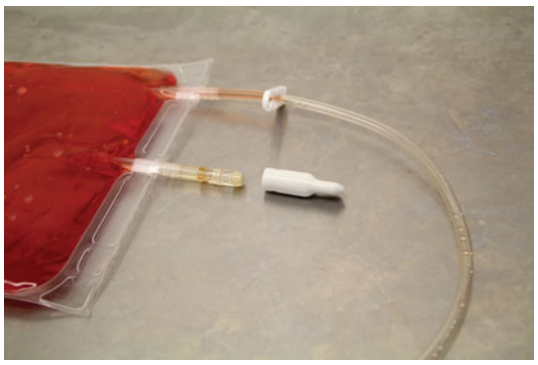


Figure 6. Placement of the Corning cell culture bag in an incubator.



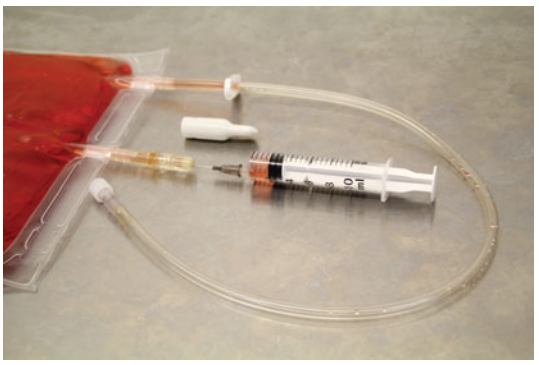


Figure 7. Sampling from the Corning cell culture bag.