Corning® Elplasia® 48K Vessel

Guidelines for Use

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Introduction

Corning Elplasia technology enables the straightforward generation of large quantities of uniform spheroids under one culture condition. The proprietary technology incorporates a gas-permeable polystyrene film, novel microcavity geometry, and Corning Ultra-Low Attachment (ULA) surface for the generation and culture of large-scale 3D cultures.

The Corning Elplasia 48K vessel incorporates the same microcavity technology as the Corning Elplasia 12K flask and 12K open well plate to generate approximately 48,000 spheroids of similar size and shape in one vessel. The three-port design provides a dedicated "Harvest/Media Exchange" port with a flat landing area and liquid diverter intended to minimize spheroid disruption during liquid exchange steps, and "Access" ports for seeding, sampling, and air flow during harvest (Figure 1). The microcavity geometry allows spheroids to remain in place during medium exchange steps without compromising full recovery at harvest/collection time.

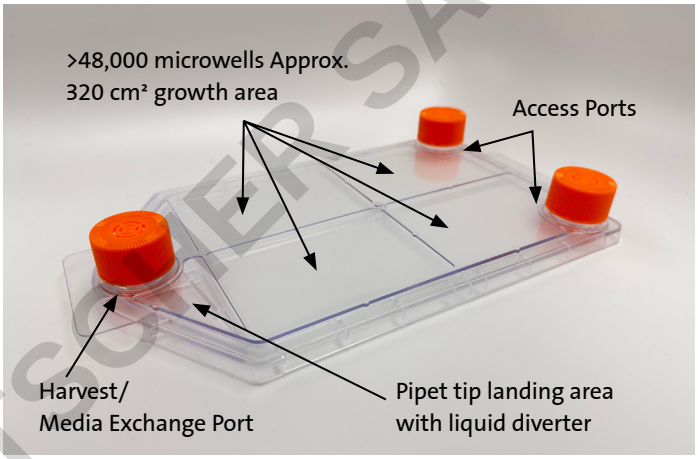


Figure 1. Corning Elplasia 48K vessel.

Some optimizations of cell culture conditions and handling will be required depending upon cell type, seeding density, and desired culture time. **It is highly recommended to review this entire document prior to use.**

Materials

- Corning Elplasia 48K vessel (Corning 9920)
- Wetting agent (0.2 µm sterile filtered 35% to 70% ethanol in water)
- Cell culture grade water (Corning 21-031-CM)
- 1X Dulbecco's Phosphate Buffered Saline (DPBS; Corning 25-055-CV)
- Single cell suspension
- Cell culture medium
- 70 µm cell strainer (Corning 431751)

Procedure

Pre-wetting the Microcavity Surface

To ensure cells settle evenly into every microcavity, trapped air needs to be displaced from the microcavity structures. To do so the surface should be pre-wetted with wetting agent (0.2 µm sterile filtered 35% to 70% ethanol (EtOH) solution) prior to seeding cells.

Once the surface has been wetted out (trapped air displaced), the vessel is ready for use. The vessel can be used immediately or can be stored temporarily in a biosafety cabinet or cell culture incubator with the final DPBS rinse until cells are ready for seeding. If the microcavity surface is allowed to dry, the wetting procedure will need to be repeated.

Work under sterile conditions and use best aseptic cell culture techniques.

1. Remove the Elplasia 48K vessel from the packaging, the bottom protective tray can stay in place or be removed.
2. Loosen all three port caps. Place the 48K vessel with the Harvest/Media Exchange port area lifted (place a 10 mL pipet under the area to lift). Dispense 40 mL of wetting agent through one or both Access ports.
3. After addition of the wetting agent, gently bring the vessel to a flat position to allow the solution to fully distribute across the microcavity surface.

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- As the wetting agent displaces the trapped air, the microcavities will appear optically clear. Microcavities with trapped air will appear opaque, cells cannot settle into those microcavities.
- Gentle agitation/swirling or tapping of the sides and edges of the vessel may be required to fully wet out surface. Avoid excessive splashing of the wetting agent.

4. Once the surface is fully wetted out (trapped air is displaced) aspirate/remove the wetting agent (recommend using the Access port).
   4.1. Lift Harvest/Media Exchange port area to drive liquid towards the Access ports for full liquid removal.
5. Add 50 mL of cell culture grade water to the vessel to rinse residual wetting agent from microcavities. Gently agitate/swirl the vessel to fully rinse the microcavity surface. Repeat aspirate/removal of liquid step.
6. Repeat the rinse steps two additional times using 50 mL of 1X DPBS to remove residual traces of the wetting agent.

Seeding Cells

Optimal seeding densities depend on factors such as cell type, culture duration, and the desired size of spheroids at the time of assessment.

Work under sterile conditions and use best practice aseptic cell culture techniques.

Option 1

This method allows all cells to settle into microcavity structures.

1. Prepare cell suspension at the desired seeding density in 40 mL complete cell culture medium.
   - Microcavity density is approximately 48,640 microcavities per vessel (152 microcavities per cm^2 x 320 cm^2 microcavity growth area).

     Example: If seeding 800 cells per microcavity; 800 cells x 48,640 microcavities = 38.9 million cells per vessel. Prepare 40 mL single cell suspension at 9.73e5 cells per mL.
   - Recommend using 70 µm cell strainer to ensure single cell suspension.
2. Place the 48K vessel in incubation position (the bottom protective tray can stay in place or be removed), add 40 mL of cell suspension through either both or one access port. Using the Access ports for seeding minimizes the number of cells that can land/settle on the flat section of the Harvest/Media Exchange port area.
   - Gently swirl/rock the vessel to distribute cell suspension evenly throughout the surface. To ensure that all cells settle into microcavity structures the liquid level should reach just below the flat section of the Harvest/Media Exchange port area.
   - Limit tilting or swirling motion to avoid getting cells on the flat portion of the Harvest/Media Exchange port area.
3. Leave vessel undisturbed for 10 to 15 minutes to allow cells to settle into microcavities. Once cells have settled, the vessel must remain in the incubation position.
   - Moving the vessel to an upright position will cause the cells to float out of the microcavities.
   - Care should be taken when handling the vessels to minimize liquid movement.
4. Use the Harvest/Media Exchange port to gently add fresh culture medium to bring volume to desired culture volume. Optimal culture volume will depend on cell type and feeding schedule:
   - 65 to 100 mL volume range recommended for short-term (no-media exchange/minimal handling) cultures.
   - 250 mL (full vessel) volume recommended for long-term cultures, cultures that require multiple media exchanges, and handling.

Helpful Hints:

- Using the Harvest/Media Exchange port for media addition prevents disruption of cells settled in microcavities under the Access ports. It also helps rinse down any cells that may have landed on the flat area under the Harvest/Media Exchange port.
- Liquid addition at high speeds may cause displacement/disruption of settled cells. It is recommended to set the pipettor at the lowest possible speed setting when dispensing liquid into vessel.

5. Keeping the vessel in the incubation position, gently transfer to the incubator. The bottom protective tray can stay in place or be removed during incubation.
   - Media level should fully cover the microcavity growth area and be evenly distributed throughout the vessel.
   - Ensure the incubator shelves are leveled to maintain uniform liquid distribution in the vessel.

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Option 2

This method is quicker and requires less handling, however it allows for cells to settle outside of the microcavities.

1. Prepare cell suspension at the desired seeding density and culture media volume:
   - Microcavity density is approximately 48,640 microcavities per vessel (152 microcavities per cm^2 in 320 cm^2 microcavity growth area).
   - Minimum working volume is 65 mL (recommended for short-term cultures). Maximum working volume of 250 mL will fill the vessel (recommended for long-term cultures/cultures that require multiple feedings). Optimal culture volume will depend on cell type, feeding schedule and extent of culture.
2. Place the 48K vessel in the incubation position, the bottom protective tray can stay in place or be removed. Dispense cell suspension through any port.
   - If filling vessel completely (250 mL):
     - Loosen all three port caps to allow for air to escape.
     - Port area used for dispensing should be lifted (the height of a10 mL pipet) to allow air to escape as the vessel fills.
     - Media volume should not go into port openings. Recap all ports tightly after fill.
3. Keeping the vessel in the incubation position, gently transfer to the incubator. The bottom protective tray can stay in place or be removed during incubation.

NOTES:

- Cultures should be left undisturbed for at least 24 hours to prevent disruption of initial spheroid formation.
- Vessels can be stacked during incubation but should be handled individually during manipulation. **Moving/transporting vessels while stacked is not recommended.**
- Once seeded, the vessel must remain in the incubation position. Moving the vessel to the upright position will cause the cells/spheroids to dislodge from the microcavities and should be avoided until ready to harvest.
- If using lower culture volume (65 to 100 mL), care should be taken when handling vessels to minimize liquid motion or excessive liquid slushing.
- Liquid addition at high speeds may cause displacement/disruption of the cells/spheroids in the microcavities. It is recommended to set the pipettor at the lowest possible speed when dispensing into the vessel.

Medium Exchange

The microcavity geometry and the Elplasia 48K vessel design allows for full medium exchange with roughly 10 mL residual volume (approx. 0.2 µL per microcavity). For cultures that require multiple media exchanges it is recommended to use 250 mL culture volume to minimize disruption of spheroids during handling.

For medium exchanges, use the Harvest/Media Exchange port landing area, which has a liquid diverter feature for gentle flow of medium into and out of the vessel. Lifting the Access port end of the vessel 3 to 4 degrees helps drive the liquid towards the Harvest/Media Exchange port for full liquid removal. Keeping the area lifted during media addition helps slow the flow of liquid into the vessel. Minimize the lift to no more than 4 degrees to prevent disruption of the spheroid cultures. An item such as a 10 mL pipet can be used to lift the vessel to the recommended height/angle (2.5 degrees) during medium exchange steps.

Work under sterile conditions and use best practice aseptic cell culture techniques.

1. Keeping the vessel in the incubation position, transfer to the biological safety cabinet, then loosen all three caps.
2. Lift the Access port section by placing a 10 mL serological pipet under the area.
   - If the bottom protective tray is kept in place, place the pipet underneath the tray to lift the vessel.
3. Use the flat feature of the Harvest/Media Exchange port for placement of the aspirating pipet tip. Aspirate/remove spent medium.
   - The Elplasia 48K vessel design allows for full or desired amount of liquid removal.
4. To dispense medium, set the pipettor to the lowest possible dispensing speed. Use the flat feature of the Harvest/Media Exchange port for placement of the pipet, slowly dispense fresh culture medium into the vessel.
5. Once the medium exchange is complete, gently bring the flask back to the flat incubation position, recap/tighten all three caps, then transfer the vessel back to the incubator.

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Spheroid Collection/Harvest

Collection volume will depend on collection/harvest method, the desired final concentration and downstream application. For optimal collection, a minimal of one rinse step using at least 50 mL of 1X DPBS is recommended.

Work under sterile conditions and use best practice aseptic cell culture techniques.

Option 1

This method uses Corning Disposable Aseptic Transfer Cap (ATC; Corning 11757), for Corning CellSTACK® Culture Chambers) to aseptically transfer spheroid collection into receiving container.

1. Bring the vessel to the biological safety cabinet. If culture volume is higher than 100 mL, gently remove/aspirate spent medium leaving roughly 50 to 100 mL in vessel.
   - Lower liquid volume generates more turbulence to aid in displacement/removal of spheroids from microcavities.
2. Replace the Harvest/Media Exchange port vented cap with the ATC. Close the tubing clamp and ensure Access port caps are on tightly. Loosen the quick connect cap on the ATC tubing.
3. Swirl, shake, and rock media in the vessel to dislodge spheroids from microcavities. Once spheroids are floating in the media, quickly invert and angle the vessel to drive spheroid collection towards the Harvest/Media Exchange port area.
   - Inverting the vessel prevents spheroids from settling back into microcavity structures.
4. Remove the ATC tubing cap, place the collection container under the tubing or connect the tubing to the collection container (closed system transfer).
5. Loosen or remove the Access port caps to allow air flow in the vessel during the emptying step. Open the tubing clamp fully for fast flow of spheroid suspension out of the vessel and into the receiving container.

Helpful Hints:

- Unimpeded/fast liquid flow out of the vessel results in higher recovery.
- Leaving Access ports closed will generate a vacuum in the vessel during the emptying step leading to drop in recovery yield and possible vessel damage.

6. Close the tubing clamp, recap the ATC tubing (if not attached to closed system collection), then return the vessel to the incubation position.
7. Additional rinses may be necessary to recover all spheroids. Using a back-and-forth motion (like a windshield wiper) add 50 to 100 mL of 1X DPBS (or plain media) through the Access ports to rinse the spheroids towards the Harvest/Media Exchange port area.
8. Swirl /rock the vessel to keep spheroids in suspension, quickly invert the vessel to prevent spheroids from settling back into microcavity structures.
9. Repeat the collection step and rinse step if necessary.

Option 2

This method uses pouring to aseptically collect spheroids.

1. Bring the vessel to the biological safety cabinet. If culture volume is higher than 100 mL, gently remove/aspirate spent medium leaving roughly 50 to 100 mL in the vessel.
   - Lower liquid volume generates more turbulence to aid in displacement/removal of spheroids from microcavities.
2. Swirl, shake, and rock media in the vessel to dislodge spheroids from microcavities. Once spheroids are floating in media, lift/angle the vessel to drive spheroid suspension towards the Harvest port.
   - Make sure liquid level is below port opening.
3. Carefully remove cap and pour suspension into collection container.
   - **Recommend the use of a wide mouth collection container.**
4. Additional rinse steps will be necessary to recover all spheroids. Using a back-and-forth motion (like a windshield wiper) add 50 mL of 1X DPBS (or media) to rinse spheroids towards the Harvest/Media Exchange port area.
5. Repeat pour collection and rinse steps for full spheroid collection.

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Option 3

This method uses pipetting to recover spheroids from the vessel.

1. Bring the vessel to the biological safety cabinet. If culture volume is higher than 100 mL, gently remove/aspirate spent medium leaving roughly 50 to 100 mL in the vessel.
   - Lower liquid volume generates more turbulence to aid in displacement/removal of spheroids from microcavities.
2. Swirl, shake, and rock media in the vessel to dislodge spheroids from microcavities. Once spheroids are floating in media, lift/angle vessel to drive spheroid suspension towards the Harvest port.
3. Place a 25 to 50 mL serological pipet under the Access port area to drive and keep spheroid collection in the Harvest/Media Exchange port area.
   - Make sure the liquid level is below the port opening.
4. Use a pipet to collect spheroids.
5. Multiple rinse steps will be necessary to recover all spheroids. Use a back-and-forth motion (like a windshield wiper) through Access ports to rinse spheroids towards the Harvest/Media Exchange port area.
6. Repeat collection and rinse steps for full spheroid collection.

Technical Specifications

Approximate microcavities per vessel	48,640
Microcavity growth surface area	320 cm^2
Total surface area	392 cm^2
Top well dimensions of microcavities (diameter x depth)	850 x 650 µm
Spheroid growth area in microcavities (diameter x depth)	500 x 600 µm
Recommended pre-wet volume	40 mL
Recommended short-term culture volume	65 to 100 mL
Recommended long-term culture volume	250 mL

Ordering Information

Cat. No.	Description	Approximate Spheroids per Plate	Microcavity Size (diameter x depth)	Spheroid Growth Area (diameter x depth)	Qty/Pk	Qty/Cs
9920	Corning® Elplasia® 48K vessel	48,000	850 x 650 µm	500 x 600 µm	1	8

Complementary Products

Cat. No.	Description	Approximate Spheroids per Plate	Microcavity Size (diameter x depth)	Spheroid Growth Area (diameter x depth)	Qty/Pk	Qty/Cs
11757	Corning Disposable aseptic transfer cap for Corning CellSTACK® culture chambers	N/A	6-inch tube, female MPC Connector	N/A	1	5

Cat. No.	Description	Qty/Pk	Qty/Cs
21-031-CM	Dulbecco's Phosphate-Buffered Saline, 1X without calcium and magnesium, pH 7.0 to 7.6	6	6
25-055-CV	Cell culture grade water, 500 mL, tested to USP Sterile Water for Injection specifications	6	6
431751	Cell strainer, 70 µm, white, sterile, individually wrapped	1	50

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