Corning® Elplasia® 12K Flask

Guidelines for Use

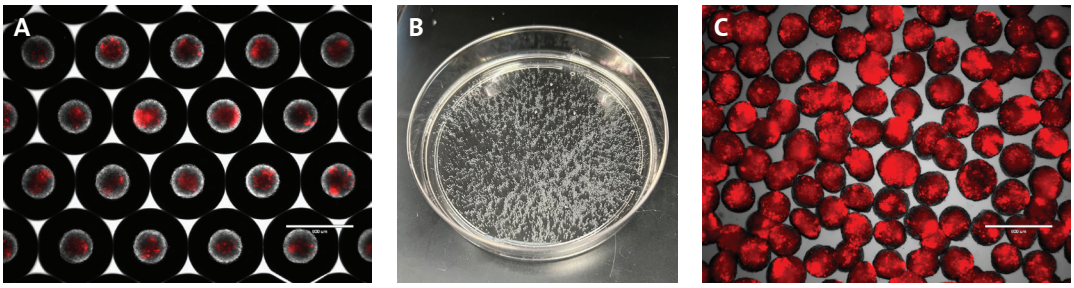
CORNING

Introduction

The Corning Elplasia 12K flask was designed to enable the straightforward generation of large quantities of uniform spheroids under one culture condition, with a gas-permeable polystyrene film flask-bottom containing 152 microcavities per cm^2 in a vessel footprint similar to that of a T-75 flask. Gravity, in conjunction with Corning Ultra-Low Attachment (ULA) surface, and rounded microcavity geometry enables the formation of approximately 12,000 spheroids of similar shape and size. The flasks are sterile and ready-to-use. A liquid diverter feature (Figure 1) allows for minimal spheroid disruption during liquid handling steps. The microcavity geometry allows spheroids to remain in place during medium exchange steps without compromising full recovery at collection/harvest time.

Some optimization 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.

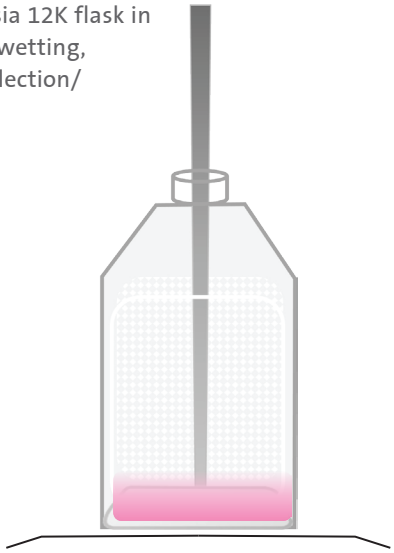
Figure 1. Arrow indicates liquid diverter feature on the Corning Elplasia 12K flask.



Representative images of 14-day HEK-293/RFP (human embryonic kidney expressing RFP, shown as red) spheroid culture in the Corning Elplasia 12K flask (A) and post-collection/harvest (B and C). Micrographs were taken with an EVOS® FL microscope with a 2X objective.

Corning Elplasia 12K Flask Working Positions

Upright position. The Corning Elplasia 12K flask in the upright position is used for pre-wetting, initial cell seeding, and spheroid collection/harvest steps.



Incubation position. The Corning Elplasia 12K flask in a flat, incubation position is maintained during incubation, when handling/transporting cultures and during medium exchange steps.

Materials

- Corning® Elplasia® 12K flask
- Wetting agent (35% to 70% ethanol in water)
- Cell culture grade water
- 1X Phosphate Buffered Saline (PBS)
- Single cell suspension
- Cell culture medium
- 70 µm cell strainer (recommended)

Procedure

Pre-wetting the Microcavity Surface

The microcavity surface should be pre-wet prior to seeding cells to ensure the cell suspension enters every microcavity. It is recommended to use a 0.2 µm sterile filtered ethanol (EtOH) solution (35% to 70% EtOH in cell culture grade water) as a wetting agent for this step.

1. Remove the Corning Elplasia 12K flask from the foil packaging, place the flask in a biological safety cabinet, the orange protective tray can be removed.
2. Working with the flask in an upright position, dispense 5 to 10 mL of wetting agent (0.2 µm filtered ≥35% EtOH) to the bottom of the flask.
3. After addition of the wetting agent, gently bring the flask down to the incubation position allowing the wetting agent to fully distribute across the microcavity surface.
4. Allow the wetting agent to enter the microcavities without assistance. As the wetting agent enters the microcavities, the microcavities will become optically clear. Should the wetting agent not enter the microcavities, trapped air will appear as opaque areas on the microcavity surface (Figure 2). If there are microcavities with trapped air, cells in suspension will not settle into those structures.

   NOTE: Gentle agitation/swirling or gently tapping the flask sides and edges may be required to fully wet out surface. Avoid splashing the wetting agent or touching the bottom of the microcavity surface directly. Pipetting the wetting reagent up and down will also help to wet out the surface.
5. Bring the flask back to the upright position, and aspirate/remove the wetting agent.
6. Add 15 mL of cell culture grade water, then bring the flask down to the incubation position to rinse the microcavity surface. Gently agitate/swirl the flask to fully cover the surface with water to remove residual wetting reagent from the microcavities. Bring the vessel back to the upright position to aspirate/remove the liquid.
7. Repeat the rinse steps two additional times using 1X PBS to remove residual traces of the wetting agent.
8. The flask can now be used to seed cells immediately or can be stored temporarily in a biosafety cabinet or cell culture incubator until cells are ready for seeding.

   NOTE: If the microcavity surface is allowed to dry, repeat the wetting procedure.

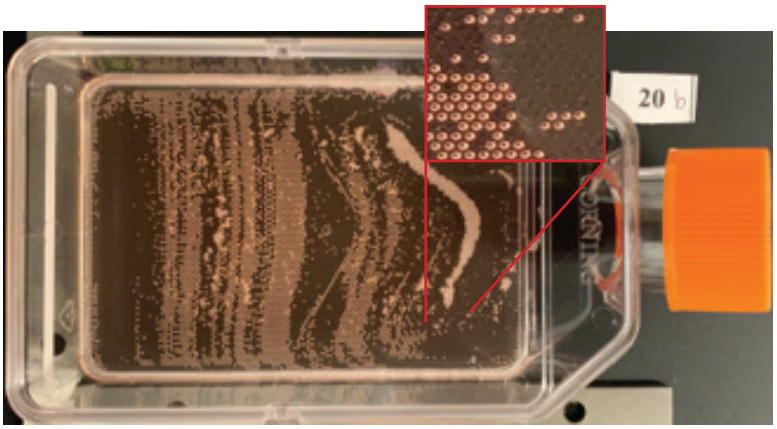


Figure 2. Example of trapped air (opaque areas) in a Corning Elplasia 12K flask if the surface is not treated with a wetting agent prior to adding medium.

Seeding Cells

Optimal seeding densities for the Corning® Elplasia® 12K flask depends on factors such as cell type, culture duration, and the desired size of spheroids at the time of assessment.

1. Prepare cell suspension at the desired seeding density in complete cell culture medium (25 to 30 mL recommended seeding volume).
   - Microcavity density is approximately 12,160 microcavities per flask (152 microcavities per cm^2 in 80 cm^2 growth area). Example: If seeding 800 cells per microcavity, use a total of 9.7 million cells (800 cells x 12,160 microcavities).
   - To ensure single cell suspension, pass cells through a 70 µm cell strainer prior to seeding.

   NOTE: Cells can be seeded directly into the flask from thaw.
2. Place the flask in the upright position, dispense the cell suspension to the bottom of the flask.
3. Keeping the flask in the upright position, transfer the flask to an appropriate cell culture incubator.
4. As you place the flask in the incubator, gently bring the flask to the incubation position. Allow the liquid to fully distribute across the microcavity surface, swirl the flask lightly to evenly distribute the cell suspension. The liquid level should fully cover the microcavity substrate and reach just below the canted neck area of the flask to ensure that all cells settle into microcavity structures (Figure 3).

NOTE: To improve ease of transport and handling do not stack the flasks more than two high. Also, cultures should be left undisturbed for at least 24 hours to prevent disruption of initial spheroid formation.

Once seeded, the flask must remain in the incubation position. Moving the vessel to the upright position may cause the cells/spheroids to dislodge from the microcavities and should be avoided. Care should be taken when handling the vessels to minimize liquid movement.

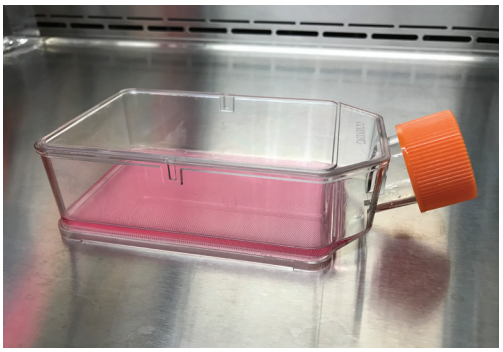


Figure 3. Medium coverage of microcavity surface at the recommended 25 mL initial seeding volume once the Corning Elplasia 12K flask is placed in the incubation position.

Medium Exchange

The microcavities of the Corning Elplasia 12K flask are deep enough to permit medium exchange with gentle handling, but not too deep that it is difficult to recover the spheroids when desired. During medium exchanges, it is important to maintain the vessel in the incubation position to prevent loss of spheroids. The canted neck and the liquid diverter feature allow for gentle flow of medium into and out of the vessel. It is recommended to use a minimum working volume of 25 mL and maximum working volume of 50 mL, however optimal final volume will depend on cell type and feeding schedule.

During medium exchange steps, lifting the back end of the flask three to four degrees drives the liquid towards the diverter feature and the canted neck to enable full liquid removal, and slows the flow of new liquid addition into the vessel. It is important to minimize the angle of the lift to no more than ten degrees when aspirating and dispensing liquids to prevent liquid from wetting out the vent cap. The orange protective tray that accompanies the flask during transit, or an item of similar height (0.25 in./ 6.35 mm) can be used to elevate the back end of the flask to the recommended three to four-degree angle during medium exchange steps.

1. Keeping the flask in the incubation position, transfer the flask to the biological safety cabinet, and loosen the cap.
2. To achieve the recommended angle position, slightly lift the back of the flask, or place an item such as the orange protective tray, a 50 mL centrifuge tube cap, or a serological pipet underneath the back end of the flask to achieve a three- to four-degree angle lift (do not lift higher than ten degrees).
   - If using the orange protective tray we recommend aligning the edge of the orange protective tray with the stacking indent features on the bottom of the flask (Figure 4).
3. To remove spent medium, place the pipet tip up against the diverter feature (as shown in Figure 5) and begin to aspirate medium out.
4. To replace medium, place the pipet tip up against the diverter feature (Figure 5) and slowly add fresh medium into the flask.
5. Once the medium exchange is complete, gently remove the angling device and bring the flask back to the flat incubation position, recap, and transfer back to the cell culture incubator.

   NOTE: The flask must remain in the incubation position during the transfer step. Moving the vessel to the upright position may result in spheroids loss from the microcavities.

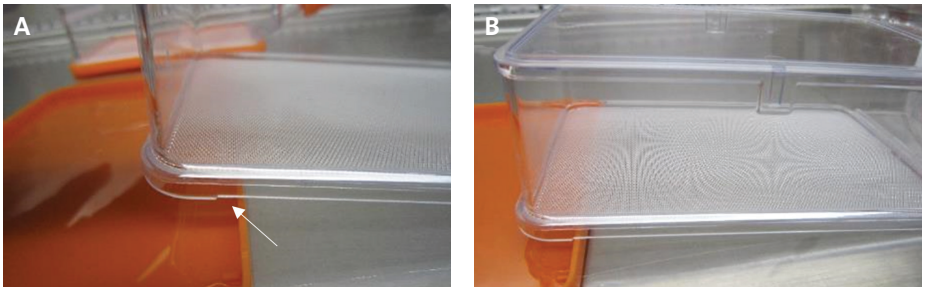


Figure 4. Recommended alignment of the Corning® Elplasia® 12K flask stacking indent features (A, arrow) on the bottom of the flask with the edge of the orange protective tray to elevate the back end of the flask to the suggested working angle during medium exchanges (B).

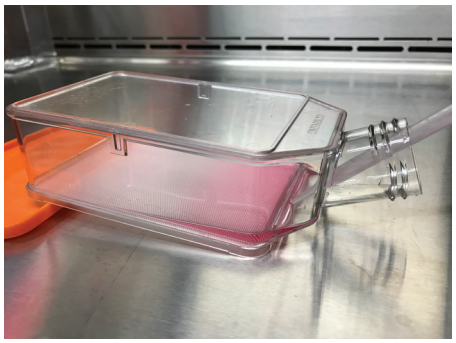


Figure 5. Recommended pipet tip position for medium exchanges.

Spheroid Collection/Harvest

Collection volume will depend on the desired final concentration and downstream application. A minimum of 15 mL of 1X PBS is recommended for collection, however, growth medium can also be used.

1. Remove spent medium from the Corning Elplasia 12K flask as described in the Medium Exchange section.
2. Add 15 mL of collection solution, and gently "tip and tap" the flask to dislodge spheroids from the microcavities.
3. Bring the flask to the upright position to release the spheroids and prevent them from settling back into the microcavities. Spheroid suspension can be collected and transferred to a separate collection container.
   - It may be necessary to perform additional rinses of the microcavity surface with fresh collection solution to recover all spheroids. A back and forth motion (like a windshield wiper) against the growth surface is recommended to rinse the spheroids out.

Spheroid Dissociation in the Flask

Depending on downstream requirements, spheroids can be collected from the Corning Elplasia 12K flask as described above for processing or dissociated into single cells directly in the vessel.

1. Remove spent medium and replace with 15 mL of 1X PBS as described in the Medium Exchange section.
   - A second 1X PBS rinse may be needed to remove a trace amount of cell culture medium.
2. Keeping the flasks in the incubation position, remove the buffer solution, then add 5 to 10 mL of dissociation reagent. Allow liquid to fully distribute across the surface, and swirl the flask lightly.
3. Incubate the spheroid cultures according to the dissociation reagent protocol for spheroid culture.
   - Spheroids will appear larger and will lose their shape once they are ready for dissociation (Figure 6).
4. Bring the flask to the upright position to release the spheroids. Pipet the suspension up and down several times to dissociate the spheroids.
5. Quench/dilute the dissociation solution with an equal volume of serum-containing growth medium, and transfer the cell suspension to a separate collection container.
   - It may be necessary to perform additional rinses of the microcavity surface for full collection. A back and forth motion (like a windshield wiper) against the growth surface is recommended to rinse the surface completely.
6. To ensure single cell suspension, cells can be passed through a 70 µm cell strainer.

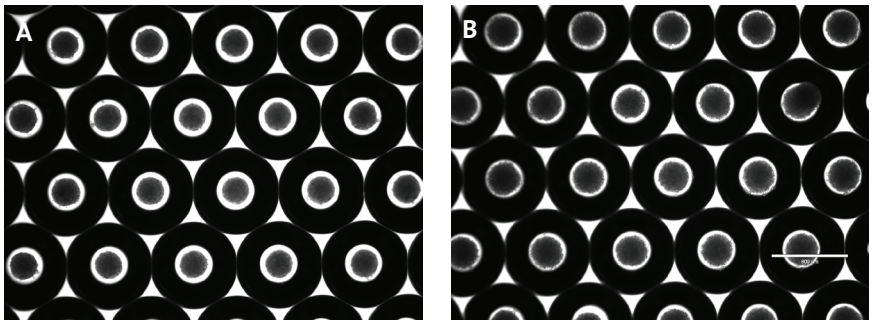


Figure 6. Seven (7)-day HT-29/GFP (human colon cancer) spheroids in a Corning® Elplasia® 12K flask before dissociation treatment (A) and post 10-minute treatment with Trypsin/EDTA solution (B). Images were taken with an EVOS FL microscope with a 2X objective.

Technical Specifications

Approximate number of microcavities per flask	12,160
Growth surface area	80 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	5 to 10 mL
Recommended seeding volume	25 to 30 mL
Recommended feeding/working volume	25 to 50 mL

Ordering Information

Products may not be available in all markets.

Cat. No.	Description	Approximate Spheroids per Flask	Microcavity Size (diameter x depth)	Spheroid Growth Area (diameter x depth)	Qty/Pk	Qty/Cs
4537	Corning Elplasia 12K flask	12,000	850 x 650 µm	500 x 600 µm	1	5

Complimentary Products

Cat. No.	Description	Qty/Cs
21-040-CM	Phosphate-Buffered Saline, 1X without calcium and magnesium, pH 7.4 ± 0.1	6
25-055-CV	Cell culture grade water, 500 mL, tested to USP Sterile Water for Injection specifications	6
431751	Cell strainer, 70 µm, white, sterile, individually wrapped	50

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