Corning® Elplasia® 12K Open Well Plate

Guidelines for Use

CORNING

Introduction

The Corning Elplasia 12K open well plate was designed similarly to the Corning Elplasia 12K flask with a gas-permeable polystyrene film-bottom containing 152 microcavities per cm^2 for the straightforward bulk generation of uniform spheroids in one culture condition. However, the microplate footprint and removable lid make the Elplasia 12K plate ideal for sampling and imaging of spheroids (Figure 1). Gravity, in conjunction with the Corning Ultra-Low Attachment (ULA) surface and rounded microcavity geometry, enable the formation of approximately 12,000 spheroids of similar shape and size. Media exchange ports (Figure 2) allows for minimal spheroid disruption during liquid handling steps. The microcavity geometry allows spheroids to remain in place throughout culture without compromising accessibility/spheroid recovery at collection time. The Elplasia 12K plate is sterile and ready-to-use.

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.**

Figure 1. The Corning Elplasia 12K open well plate design incorporates standard ANSI/SLAS microplate footprint and removable lid.

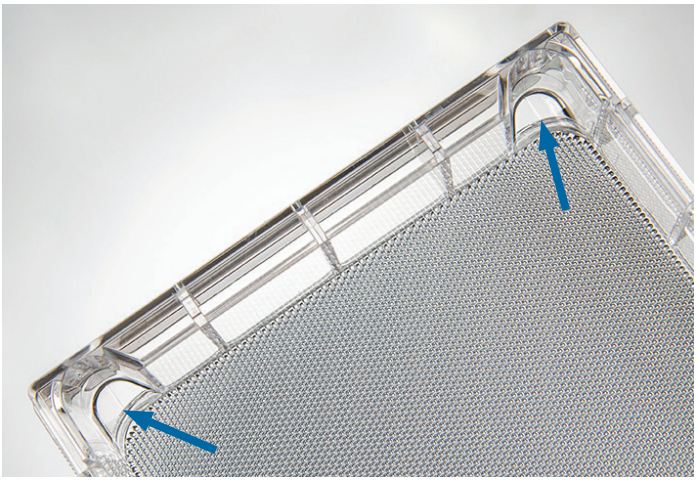

Figure 2. Image of Corning Elplasia 12K open well plate. Arrows indicate media exchange ports designed for liquid handling steps.

Materials

- Corning Elplasia 12K open well plate (Cat. No. 4547)
- Wetting agent (35-70% ethanol in water)
- Cell culture grade water
- 1X Phosphate Buffered Saline (PBS)
- Single cell suspension
- Cell culture medium

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. Alternatively, the Elplasia 12K plate can be centrifuged briefly (<3 min.) at 300 xg to drive liquid into the microcavities.

Recommended pre-wet volumes

Wetting agent: 5 to 10 mL
Centrifugation: 10 to 13 mL 1X PBS or cell culture media

Wetting Agent Method

1. Remove the Elplasia 12K plate from the primary packaging, place in a biological safety cabinet, the orange protective tray can be removed.
2. Remove the lid, and dispense 5 to 10 mL of wetting agent (0.2 µm filtered ≥35% EtOH) with a serological pipet placed in one of the media exchange ports.

3. Allow the wetting agent to fully distribute across the microcavity surface and enter the microcavities without assistance. As liquid enters the microcavities, the microcavities will become optically clear. If liquid fails to enter the microcavities, trapped air will appear as opaque areas on the microcavity surface. If there are microcavities with trapped air, cells in suspension will not settle into those structures.

   NOTE: Gentle agitation/swirling or gently tapping the Elplasia 12K plate sides and edges may be required to fully distribute the liquid across the 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.

4. Aspirate/remove the wetting agent via one of the media exchange ports.
5. Add 15 mL of cell culture grade water, and rinse the microcavity surface to remove residual wetting reagent from the microcavities. Aspirate/remove the water.
6. Repeat rinse steps two additional times using 1X PBS to remove residual traces of the wetting agent.

Centrifugation Method

1X PBS or cell culture medium can be used for the pre-wetting step. A volume of 10 to 13 mL is recommended with 300 xg centrifugation. DO NOT exceed 13 mL of liquid or RCFs higher than 500 xg or liquid can spill out of the Elplasia 12K plate during centrifugation. RCFs exceeding 1,000 xg will damage the Elplasia 12K plate. When placing the Elplasia 12K plate into the centrifuge, the media exchange ports should be facing outward (away from the rotor) to prevent liquid breaches during centrifugation.

1. Remove the Elplasia 12K plate from the primary packaging, place in biological safety cabinet, the orange protective tray can be removed.
2. Remove the lid and dispense 10 to 13 mL 1X PBS or cell culture media with a serological pipet placed in one of the media exchange ports.
3. Allow the liquid to fully distribute across the microcavity surface and enter the microcavities. Gentle agitation/swirling the Elplasia 12K plate may be required to fully distribute the liquid.
4. Replace the lid and transfer the Elplasia 12K plate to the centrifuge. Load it into the centrifuge with the media exchange ports facing outward (away from the rotor), to prevent liquid from spilling out during centrifugation.
5. Centrifuge at 300 xg for 3 minutes. Time and speed may need to be increased depending on the medium used. Do Not exceed RCF higher than 500 xg or volumes over 13 mL.
6. Remove the Elplasia 12K plates from the centrifuge, gentle agitation/swirling may be required to redistribute liquid.

NOTE: Once the microcavity surface has been pre-wet the Elplasia 12K plate is ready for seeding. Alternatively, the plate can be stored temporarily in a biosafety cabinet or cell culture incubator until ready for seeding. If the microcavity surface is allowed to dry, repeat the wetting procedure.

Seeding Cells

Optimal plating densities for the Elplasia 12K plate 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 desired seeding density in complete cell culture medium (20 to 30 mL recommended seeding volume).
   - Microcavity density is approximately 12,160 microcavities per Elplasia 12K plate (152 microcavities per cm^2).

     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 Elplasia 12K plate from thaw.
2. Aspirate any residual liquid from the pre-wetting step then dispense the cell suspension via one of the media exchange ports. Allow the cell suspension to fully distribute across the microcavity surface, and gently swirl to evenly distribute. The liquid level should fully cover the microcavity substrate but not fill the Elplasia 12K plate completely.
3. Replace the lid and transfer the Elplasia 12K plate to a cell culture incubator.

   NOTE: Cultures should be left undisturbed for at least 24 hours to prevent disruption of initial spheroid formation.

Medium Exchange

The microcavities of the Elplasia 12K plate 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 use the media exchange ports to prevent loss of spheroids. It is recommended to use a minimum working volume of 20 mL and maximum working volume of 30 mL; however optimal final volume will depend on cell type and desired feeding schedule. It is recommended to perform a media exchange at least every 3 to 4 days to avoid loss due to evaporation.

During medium exchange steps, the Elplasia 12K plate can be kept flat. However, lifting the short side of the Elplasia 12K plate opposite of the media exchange ports three-to-four degrees drives the liquid towards the media exchange ports to enable full liquid removal, and slows the flow of new liquid addition into the Elplasia 12K plate. If choosing to elevate, it is important to minimize the angle of the lift to prevent liquid from overflowing. An item of approximately 0.25 in. (6.35 mm) can be used to elevate the side of the Elplasia 12K plate to the recommended three-to-four-degree angle during medium exchange steps.

1. Transfer the Elplasia 12K plate to the biological safety cabinet and remove lid.
2. The Elplasia 12K plate can be kept flat, or to achieve the recommended three-to-four-degree angle, slightly lift the short side of the Elplasia 12K plate opposite of the media exchange ports (0.25 in./6.35 mm) or place an item such as a 50 mL centrifuge tube cap or a serological pipet underneath to achieve the desired lift (as shown in Figure 3).
3. To remove spent medium, place a serological pipet tip into one of the media exchange ports and begin to aspirate medium out.
4. To replace medium, place a serological pipet tip into one of the media exchange ports at a slight angle (as shown in Figure 4) and slowly add fresh medium into the Elplasia 12K plate.

   NOTE: If the pipet tip is placed in the media exchange port perpendicular to the Elplasia 12K plate, there is a risk of higher spheroid disturbance in the microcavities adjacent to the port during liquid dispensing.
5. Once medium exchange is complete, gently remove the angling device if utilized, and bring the Elplasia 12K plate back to a flat position, replace the lid, and transfer the Elplasia 12K plate back to the cell culture incubator.

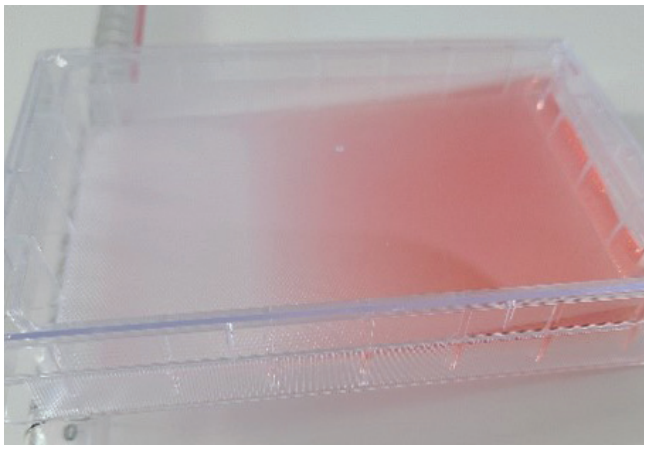

Figure 3. Recommended lift position to enable full liquid removal. Example shown using a 25 mL serological pipet.

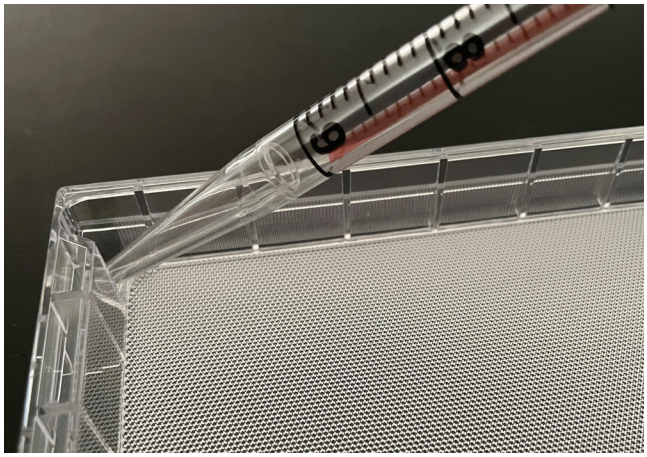

Figure 4. Recommended pipet tip position at a slight angle in one of the media exchange ports during medium addition.

Spheroid Access/Collection

The Elplasia 12K plate removable lid, enables easy access to spheroid cultures.

For individual spheroid access/ collection it is recommended to utilize a blunt end tip or needle with inner diameter of 0.381 mm (i.e., 24G blunt end needle) to individually access spheroids of interest. Using a smaller bore size may result in shearing or distortion of the selected spheroid, depending on spheroid diameter. Using a larger bore size may result in aspirating multiple spheroids from adjacent cavities.

For mass collection of spheroid cultures, the Elplasia 12K plate can be angled to approximately three-to-four degrees and gently tapped to dislodge spheroids from microcavities. Several rinse and collection steps may be required as dislodged spheroids will settle back into available microcavities.

Spheroid Dissociation

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

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 trace amounts of cell culture medium.
2. Remove buffer solution, then add 5 to 10 mL of dissociation reagent. Allow liquid to fully distribute across the surface.
3. Incubate spheroid cultures according to dissociation reagent protocol for spheroid culture.
   - Spheroids will appear larger and will lose their shape once they are ready.
4. Swirl the Elplasia 12K plate to help dislodge the spheroids, pipet the suspension up and down several times to fully dissociate spheroids.
5. Quench/dilute dissociation solution with an equal volume of serum-containing growth medium, and transfer cell suspension to a separatecollection container.
   - It may be necessary to perform additional rinses of the microcavity surface for full cell collection.
6. To ensure single cell suspension, cells can be passed through a 70 µm cell strainer.

Technical Specifications

Approximate number of microcavities per open well plate	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 (wetting agent) 10 to 13 mL (centrifugation)
Recommended seeding volume	20 to 30 mL
Recommended feeding volume	20 to 30 mL

Ordering Information

Products may not be available in all markets.

Corning® Elplasia® 12K Open Well Plate

Cat. No.	Description	Approximate Spheroids per Plate	Microcavity Size (diameter x depth)	Spheroid Growth Area (diameter x depth)	Qty/Pk	Qty/Cs
4547	Corning Elplasia 12K open well plate	12,000	850 x 650 µm	500 x 600 µm	1	5

Complementary 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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