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Expansify™ Cell Culture Methods- A549 Expansion

Based on internally validated culture conditions and representative experimental data generated using Expansify™ plates

Purpose

This culture guide provides representative conditions for the expansion and maintenance of A549 cells in Expansify™ 24- and 1-Well Plates and includes representative data generated using an alpha unit of the closed Gigacell™ bioreactor. The protocol is based on internally generated experimental data and includes representative morphology, seeding conditions, media exchange recommendations, and expected growth characteristics observed under XDemics validated laboratory conditions. Further optimization may be required based on the intended application and desired outcome.

Materials

Prior to use, review the Expansify™ Quick Start Guide and instructional video series in the resources section of the XDemics website.

- Expansify™ 24-Well Plate (Cat. No. 24W-R-01-01) or 1-Well Plate (Cat. No. 01W-R-01-01)
- Cell line: A549 (ATCC® CCL-185™).
- Single-use glass or polystyrene Pasteur pipettes are required for fluid handling (e.g., CELLTREAT® Polystyrene Pasteur Pipette, Cat. No. 229280).
- Culture medium: DMEM High Glucose (Gibco, Cat. No. 11965092) supplemented with 10% Fisherbrand™ Research Grade Fetal Bovine Serum, Canadian Sourced (Cat. No. FB12999102).
- Phosphate-buffered saline (PBS), Ca^{2+}/Mg^{2+}-free (Gibco, Cat. No. 10-010-023)
- EDTA (0.5 M), pH 8.0, RNase-free (Invitrogen™, Cat. No. AM9260G)
- Dissociation reagent: TrypLE™ Express Enzyme (1×), no phenol red (Gibco, Cat. No. 12604013)
- Extracellular matrix (ECM) coating reagent: Vitronectin (VTN-N) Recombinant Human Protein, truncated (Gibco, Cat. No. A400458)
- Wide-bore P1000 pipette tip (recommended)
  NOTE: Equivalent products from other manufacturers may be substituted for the culture medium, FBS, TrypLE™ cell dissociation reagent, vitronectin coating reagent, and wide-bore P1000 pipette tips listed above. All other materials should be used as specified, including the listed manufacturer and catalog number.

Experimental Procedure

Gigacell™ operating guidance: This protocol includes representative performance data generated using an alpha unit of the closed Gigacell™ system. Because Gigacell™ incorporates format-specific fluid paths, connections, and handling procedures, detailed operating instructions are not included in the step-by-step workflow presented below. For current guidance on Gigacell™ setup, fluid handling, medium exchange, and cell harvest, please contact the XDemics Technical Support team. Comprehensive device-specific instructions will be provided in the Gigacell™ User Manual upon product release.

Day -1: ECM Coating

1. Prepare ECM-coated Expansify™ plates according to **Expansify™ Technical Note - Extracellular Matrix (ECM) Coating of Expansify™ Plates**.

Day 0: Culture Initiation

1. Aspirate the ECM coating solution according to the Expansify™ plate format:
    a. 24-Well Plate: Aspirate the coating solution using the 0.25 mL SureShelves™.
    b. 1-Well Plate: Aspirate the coating solution through SureShelves™ A. The plate may be tilted slightly toward the SureShelves™ to facilitate removal of the remaining coating solution.
2. Immediately add complete culture medium:
    a. **24-Well Plate:** 1 mL per well.
    b. **1-Well Plate:** 35 mL per plate.
3. Inspect the bottom of the niches for trapped air bubbles. If air bubbles are visible, centrifuge the plate at 200 × g for 1 minute using an appropriate Expansify™ plate or deep-well plate as a balance. Confirm that the niches are fully wetted before proceeding.
    NOTE: Following ECM coating, the culture surface is typically already wetted, and centrifugation may not be necessary. However, occasional air bubbles may remain or become trapped within the niches. These bubbles can prevent complete filling of the niche and reduce the surface area available for cell attachment and growth.
4. Seed A549 cells at 2.5 × 10^4 cells/cm^2 into the desired wells of the Expansify™ 24-Well Plate or 1-Well Plate.
5. Adjust the final culture volume to:
    a. **24-Well Plate:** 4.5 mL per well.
    b. **1-Well Plate:** 100 mL per plate.
6. Following seeding, gently homogenize the cell suspension to promote uniform cell distribution and settling throughout the culture niches.
7. Monitor and image cultures as needed throughout the culture period (e.g., Days 1–8).

Days 5, 6 and 7: Medium Exchange

Perform complete medium exchanges on Days 5, 6, and 7:

a. **24-Well Plate:** Keep the plate level on the work surface and aspirate the spent medium using the 0.25 mL SureShelves™. Add 4.5 mL of fresh complete medium using the 0.25 mL or 1 mL SureShelves™.
b. **1-Well Plate:** Keep the plate level and remove spent medium through SureShelves™ A. Once most of the medium has been removed, the plate may be tilted approximately 5° toward the shelf to facilitate removal of the remaining ~20 mL. Add 100 mL of fresh complete medium through SureShelves™ A.
TIP: For the 1-Well Plate, use the same designated locations for medium addition and aspiration during each exchange to minimize disruption to the culture. For example, consistently add medium from one side and aspirate from the opposite SureShelves™.

Day 8: Cell Harvest

1. Harvest cells according to the Expansify™ plate format being used:
   a. **24-Well Plate:**
      i. **Wash the culture:** Keep the plate level on the work surface. Aspirate the spent medium using the 0.25 mL SureShelves™. Add an excess volume of wash buffer, > 2 mL per well, such as 1× PBS (Ca^{2+}/Mg^{2+}-free) supplemented with EDTA to a final concentration of 5 mM, using the 0.25 mL or 1 mL SureShelves™, then aspirate the wash buffer using the 0.25 mL SureShelves™.
      ii. **Initiate cell dissociation:** Add TrypLE™ Express Enzyme (1×) to each well to be harvested according to the anticipated cell number, estimated based on the expected doubling time and duration of culture:
         1. **≤ 2×10^6 cells/well:** 1 mL
         2. **> 2×10^6 to < 4×10^6 cells/well:** 1–2 mL
         3. **> 4×10^6 cells/well:** 2–3 mL

      Gently mix up and down 2–3 times using a P1000 wide-bore pipette tip to distribute the enzyme throughout the well, then incubate at 37°C and 5% CO_2 for 7–10 minutes.
      iii. **Assess dissociation:** Remove the plate from the incubator and gently mix the culture using a P1000 wide-bore pipette tip. Examine the cells under a microscope to assess dissociation. If a uniform single-cell suspension has not been achieved, return the plate to the incubator for an additional 7–10 minutes, then repeat the gentle mixing step. Avoid excessive mixing or mechanically forcing dissociation, as this may increase shear stress and reduce cell viability.
      NOTE: The recommended TrypLE™ Express Enzyme (1×) volumes and incubation conditions are based on XDemics' internal validation. Under these conditions, a uniform single-cell suspension should typically be achieved within a cumulative incubation time of approximately 30 minutes at the highest cell densities evaluated in Expansify™, with shorter incubation times generally sufficient at lower cell densities. If additional

dissociation is required, repeated incubation and gentle mixing steps may be performed, and additional TrypLE™ Express Enzyme (1×) may be added as needed. Additional incubation beyond 30 minutes should be minimized to limit unnecessary enzymatic exposure and preserve cell integrity and viability.

iv. **Collect the cells:** Collect the resulting cell suspension for downstream processing, analysis, or further expansion.
NOTE: TrypLE™ Express Enzyme (1×) does not require neutralization. Following cell dissociation, complete culture medium may be added to dilute residual enzyme activity as appropriate for downstream processing. Record the final harvest volume prior to downstream processing.

b. **1-Well Plate:**

i. **Wash the culture:** Keep the plate level on the work surface. Aspirate the spent medium through the right-side SureShelves™ A. Once most of the medium has been removed, the plate may be tilted approximately 5° toward the aspiration side to facilitate removal of the remaining liquid. Return the plate to a level position, then add an excess volume of wash buffer, >40 mL, such as 1× PBS (Ca^{2+}/Mg^{2+}-free) supplemented with EDTA to a final concentration of 5 mM, through the left-side SureShelves™ A. Aspirate the wash buffer through the right-side SureShelves™ A. Once most of the wash buffer has been removed, tilt the plate approximately 5° toward the aspiration side to remove as much residual wash buffer as possible and minimize dilution of the dissociation reagent used in the subsequent step.
TIP: For the 1-Well Plate, use the same designated locations for medium addition and aspiration during each exchange to minimize disruption to the culture. For example, consistently add medium from one side and aspirate from the opposite SureShelves™.

ii. **Initiate cell dissociation:** Add TrypLE™ Express Enzyme (1×) to the plate to be harvested according to the anticipated cell number, estimated based on the expected doubling time and duration of culture:
   1. **≤ 7×10^7 cells:** 35 mL
   2. **> 7×10^7 to < 1.4×10^8 cells:** 35–70 mL
   3. **≥ 1.4×10^8 cells:** 70–100 mL

Gently mix using a 25 mL or 50 mL serological pipet to distribute the enzyme throughout the culture, then incubate at 37°C and 5% CO_2 for 7–10 minutes.

iii. **Assess dissociation:** Remove the plate from the incubator and gently mix the culture using a 25 mL or 50 mL serological pipet. Examine the cells under a microscope to assess dissociation. If a uniform single-cell suspension has not been achieved, return the plate to the incubator for an additional 7–10 minutes, then repeat the gentle mixing step. Avoid

excessive mixing or mechanically forcing dissociation, as this may increase shear stress and reduce cell viability.

NOTE: The recommended TrypLE™ Express Enzyme (1×) volumes and incubation conditions are based on XDemics' internal validation. Under these conditions, a uniform single-cell suspension should typically be achieved within a cumulative incubation time of approximately 30 minutes at the highest cell densities evaluated in Expansify™, with shorter incubation times generally sufficient at lower cell densities. If additional dissociation is required, repeated incubation and gentle mixing steps may be performed, and additional TrypLE™ Express Enzyme (1×) may be added as needed. Additional incubation beyond 30 minutes should be minimized to limit unnecessary enzymatic exposure and preserve cell integrity and viability.

iv. **Collect the cells:** Collect the resulting cell suspension for downstream processing, analysis, or further expansion.

NOTE: TrypLE™ Express Enzyme (1×) does not require neutralization. Following cell dissociation, complete culture medium may be added to dilute residual enzyme activity as appropriate for downstream processing. Record the final harvest volume prior to downstream processing.

Performance Summary

Data represent three independently harvested wells for the Expansify™ 24-Well Plate (n = 3), one Expansify™ 1-well device (n = 1), and one Gigacell™ device (n = 1). All cultures were destructively harvested at their designated harvest time points, with cell viability assessed at all time points by AOPI staining using the Cellaca MX cell counter with the built-in "Cell Lines" assay.

24-Well Plate: A549 cells expanded robustly in the Expansify™ 24-Well Plate, reaching 4.80 × 10^6 cells per well with 93% viability at harvest. The observed doubling time of 29.27 hours and area-normalized yield of 2.40 × 10^6 cells/cm^2 establish the 24-Well Plate as a representative small-scale format for A549 expansion and process evaluation.

1-Well Plate: Expansion in the Expansify™ 1-Well Plate increased total cell output to 1.38 × 10^8 cells while maintaining growth kinetics comparable to the 24-well format. Cells exhibited a doubling time of 30.50 hours, an area-normalized yield of 1.96 × 10^6 cells/cm^2, and 90% viability at harvest, supporting translation of the culture to a larger Expansify™ format.

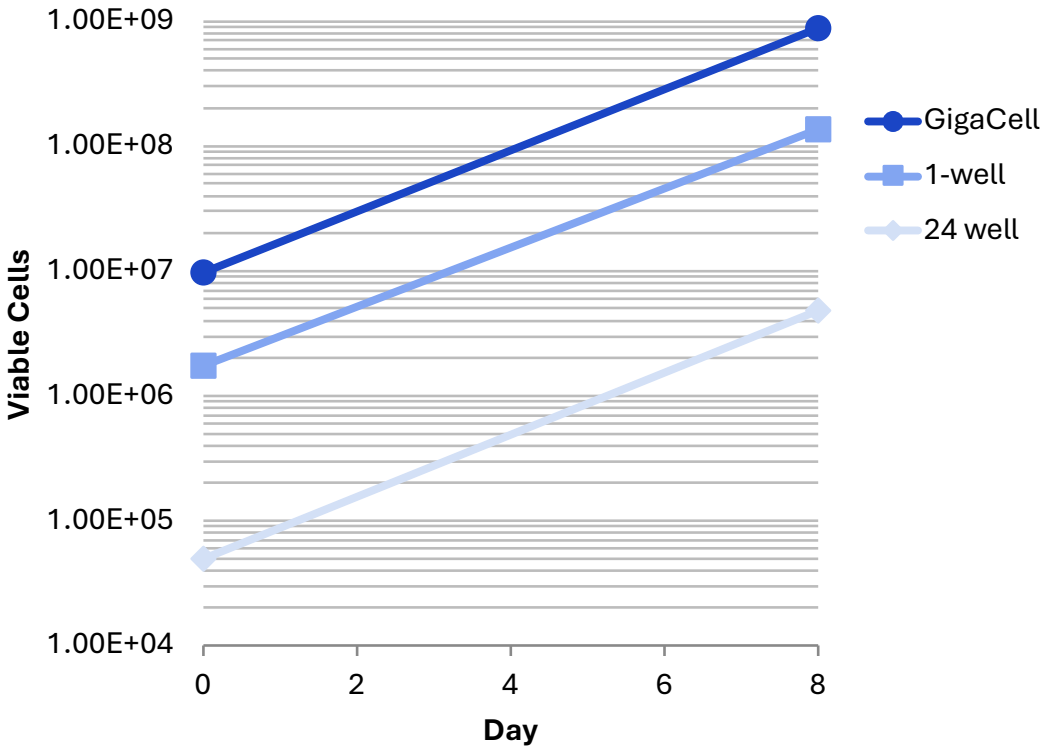
Gigacell™: A549 expansion was also evaluated in an alpha unit of the closed Gigacell™ system. The culture produced 8.69 × 10^8 cells with 92% viability at harvest, while maintaining a doubling time of 29.49 hours and an area-normalized yield of 2.25 × 10^6 cells/cm^2. These results demonstrate maintenance of comparable growth kinetics and cell yield per unit culture area at the largest format evaluated.

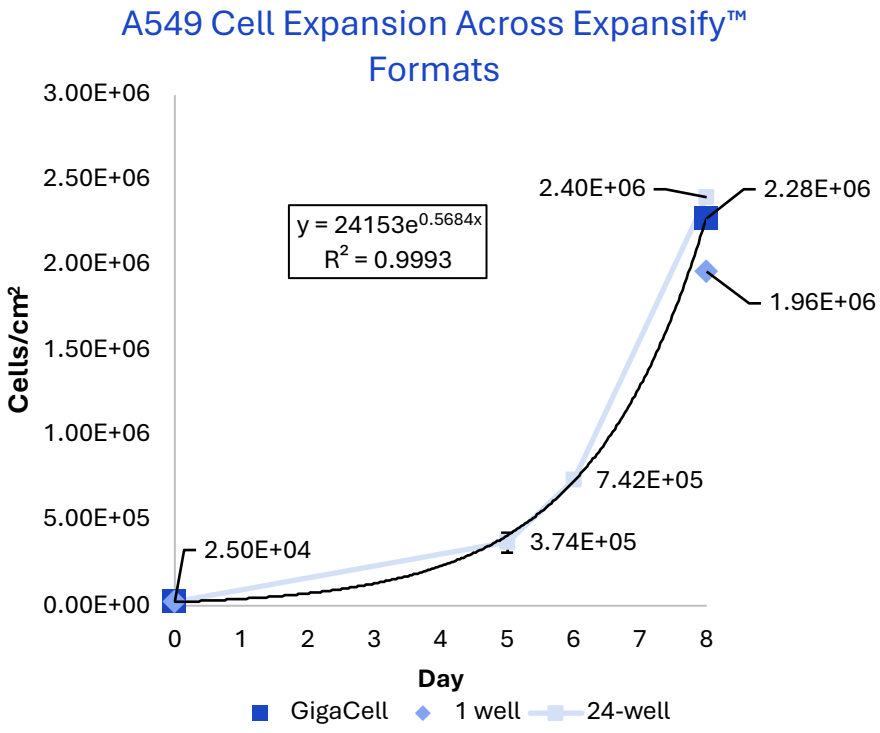
Cross-Format Performance: Across the Expansify™ 24-Well Plate, 1-Well Plate, and Gigacell™ formats, A549 cells maintained closely aligned growth kinetics as culture surface area increased from 2 cm^2 to 386 cm^2. Doubling times ranged from 29.27 to 30.50 hours, while area-normalized yields remained between 1.96 × 10^6 and 2.40 × 10^6 cells/cm^2 and harvest viability remained 90–93%. Total cell yield increased from 4.80 × 10^6 cells per well in the 24-Well Plate to 8.69 × 10^8 cells in Gigacell™, supporting consistent cell expansion across Expansify™ device formats.

Table 1. Cell expansion performance across Expansify™ device formats.

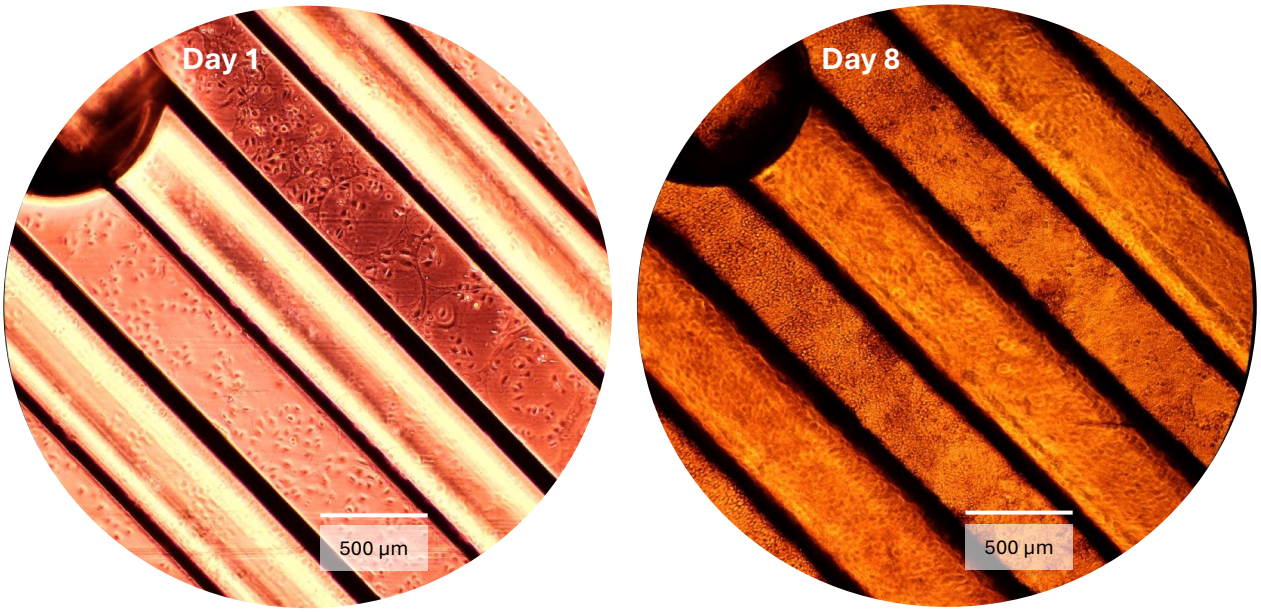
Viability and yield were determined by AOPI staining on the Cellaca MX using the built-in "Cell Lines" assay.

Device	Surface Area (cm^2)	Avg. Doubling Time (hrs)	Total Cell Yield	Yield (cells/cm^2)	Viability at Harvest (%)
24-well (n=3 wells)	2	29.27	4.80 × 10^6	2.40 × 10^6	93
1-well (n=1 device)	70	30.50	1.38 × 10^8	1.96 × 10^6	90
Gigacell™ (n=1 device)	386	29.49	8.69 × 10^8	2.25 × 10^6	92


1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
Viable Cells
0
2
4
6
8
Day
GigaCell
1-well
24 well


A549 Cell Expansion Across Expansify™ Formats
3.00E+06
2.50E+06
2.00E+06
1.50E+06
1.00E+06
5.00E+05
0.00E+00
Cells/cm²
y = 24153e^0.5684x
R² = 0.9993
2.40E+06
2.28E+06
1.96E+06
7.42E+05
3.74E+05
2.50E+04
0
1
2
3
4
5
6
7
8
Day
GigaCell
1 well
24-well

A549 Expansion in Expansify™ 24-Well Plate



Representative images of A549 cells exhibiting strong adherent morphology 1 day post-seeding. Following initial localization within the culture niches, A549 cells underwent rapid expansion and progressively populated the oxygenating surfaces over the 8-day culture period. Images were acquired using an ECHO Revolve microscope at 4× magnification.