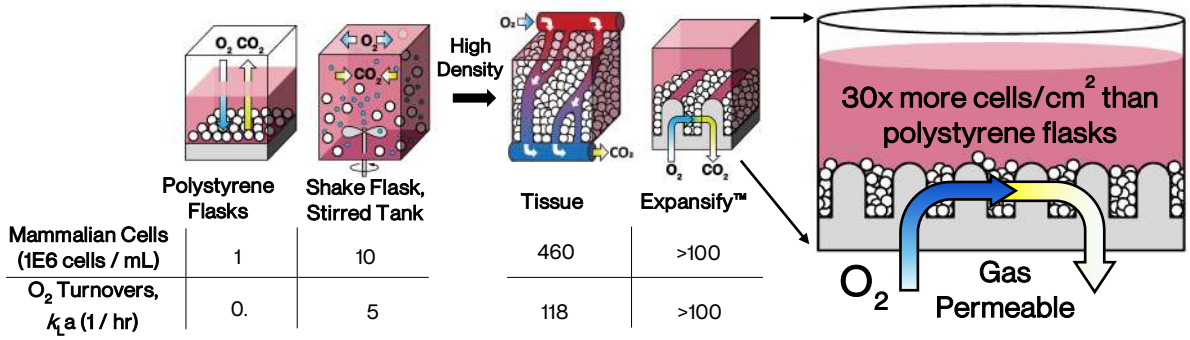
XDemics

Limitless Cell Culture

Introduction

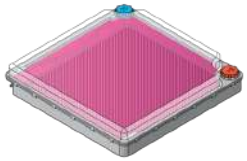
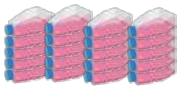
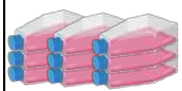
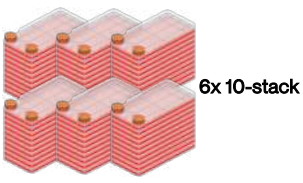
Expansify™ respiring cultureware features a series of gas-permeable ridges and grooves engineered to enhance oxygen transfer, supporting the intensified culture of adherent, spheroid, and suspension cell types. The platform's proprietary architecture is derived from a repeating unit-cell geometry (Figure 1), which establishes a uniform and well-defined microenvironment across all formats within the Expansify™ product family. This replicative design ensures consistent oxygenation, nutrient exchange, and hydrodynamic conditions, resulting in reproducible cell growth behavior and scalable product yields across the Expansify™ portfolio (Table 1). This document serves to provide representative examples of culture conditions, seeding densities, and experimental outcomes to guide prospective users to discover what is achievable with Expansify™.

Figure 1: Traditional cell culture systems are fundamentally limited by inadequate oxygen transfer ($k_L a$), which constrains cell density, viability, and functional performance. Expansify™ addresses this limitation through a biomimetic design that enables efficient, tissue-level gas exchange, closely resembling the oxygen delivery mechanisms found in living organisms.



	Polystyrene Flasks	Shake Flask, Stirred Tank	Tissue	Expansify™
Mammalian Cells (1E6 cells / mL)	1	10	460	>100
O_2 Turnovers, $k_L a$ (1 / hr)	0.	5	118	>100

Table 1: Full Expansify™ family of manually operated cell culture devices, their approximate cellular output based on HEK293T cells grown in vitronectin coated devices, and equivalent polystyrene-based counterparts for reference.

Yield (per cm²)	96-Well Launch 2026	24-Well Available Now	6-Well Launch 2027	Single-Well Available Now	Gigacell™ Tray Launch 2026 (Beta Units H1)
Expansify™: >3E6 cells					
Surface Area	0.32 cm²/ well	2 cm²/ well	8 cm²/ well	70 cm²/ well	387 cm²
Total Cells (e.g. HEK293T)	0.5 million/ well	20 million/ well	80 million/ well	500 million/ plate	>3.5 billion/ tray
Polystyrene: ~1E5 cells ~100x Yield / cm² Versus Polystyrene	 16 x 6-well plates	 24 x T-75	 6-9 x T-225	1 x 10-Stack  3 x 1720 cm² flask	 6x 10-stack

Contents

Expansify Example Experiment: K562 (Suspension cell line)

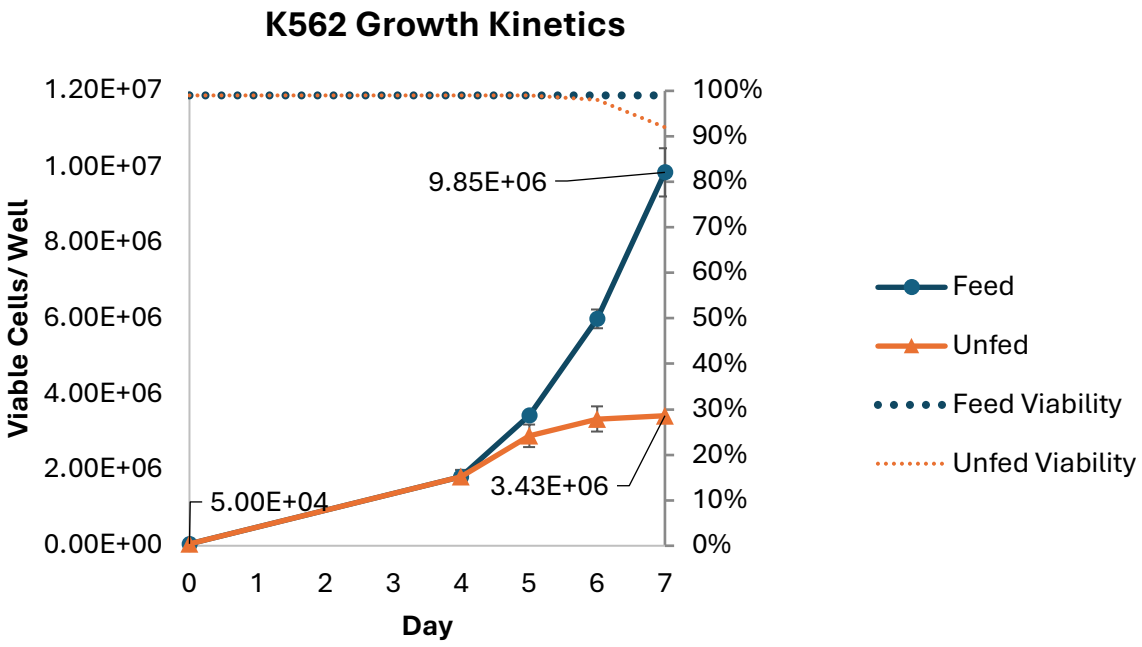
Purpose: Evaluate growth kinetics of fed vs unfed suspension K562 cells in Expansify™ 24-well plates over one week.

Experimental set-up:

- *Review quick start guide + XDemics instructional video series.*
- *K562 cell line: ATCC catalog # CCL-243*
- D0: Introduce 5×10^4 total cells per well and fill to max media fill on D0.
    - Blue fed cohort received full media exchanges (4.5 mL) on days 4-6.
    - Orange unfed cohort grew in the initial 4.5 mL of media on D0 and received no further media exchanges over the duration of the experiment.
- Media: RPMI 1640 + 10% FBS (Gibco).
- Duplicate wells counted (trypan exclusion) and averaged for each timepoint.

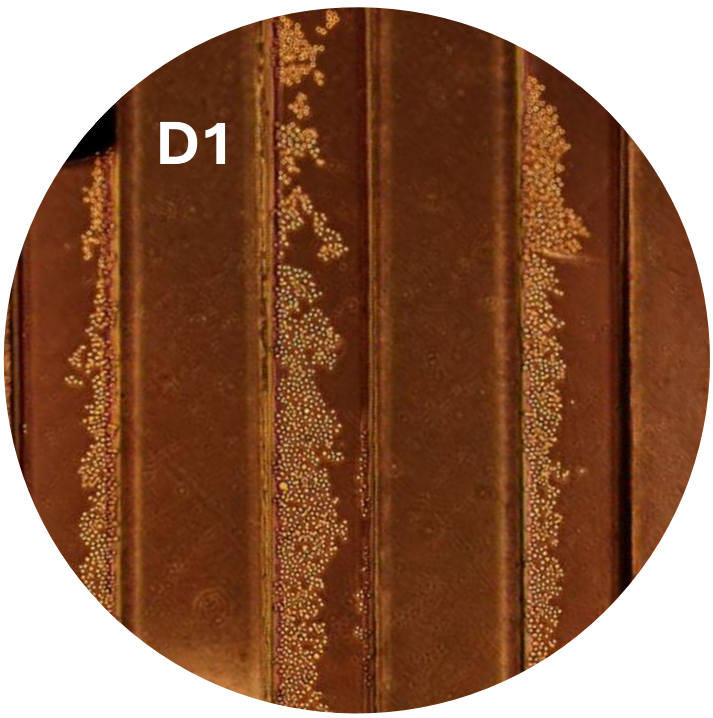
Results:

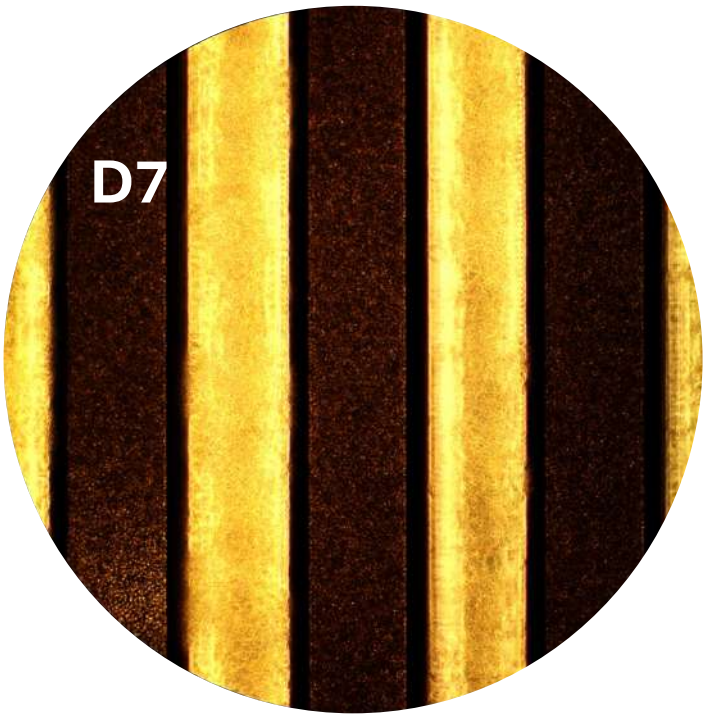
- Feed cohort: experienced exponential growth characteristics (average doubling time 20.36 hours) with high viability (> 98% at all timepoints; Trypan exclusion cell counting). Culture expanded >190 fold over 7 days without passaging.
    - 18 mL of media used over duration of culture (4.5 mL initial fill + 4.5 mL media exchanges on days 4,5, and 6).
    - Reached peak cell density of 9.85E6 cells/ well by Day 7.
- Unfed cohort: experienced exponential growth until day 4 of culture, then entered plateau phase for duration of culture (68-fold expansion). Viability remained >90% even in unfed conditions.



Example Images in Expansify™ 24Well Plate

Taken on ECHO Revolve 4X magnification





Expi293PRO (Suspension HEK cell line)

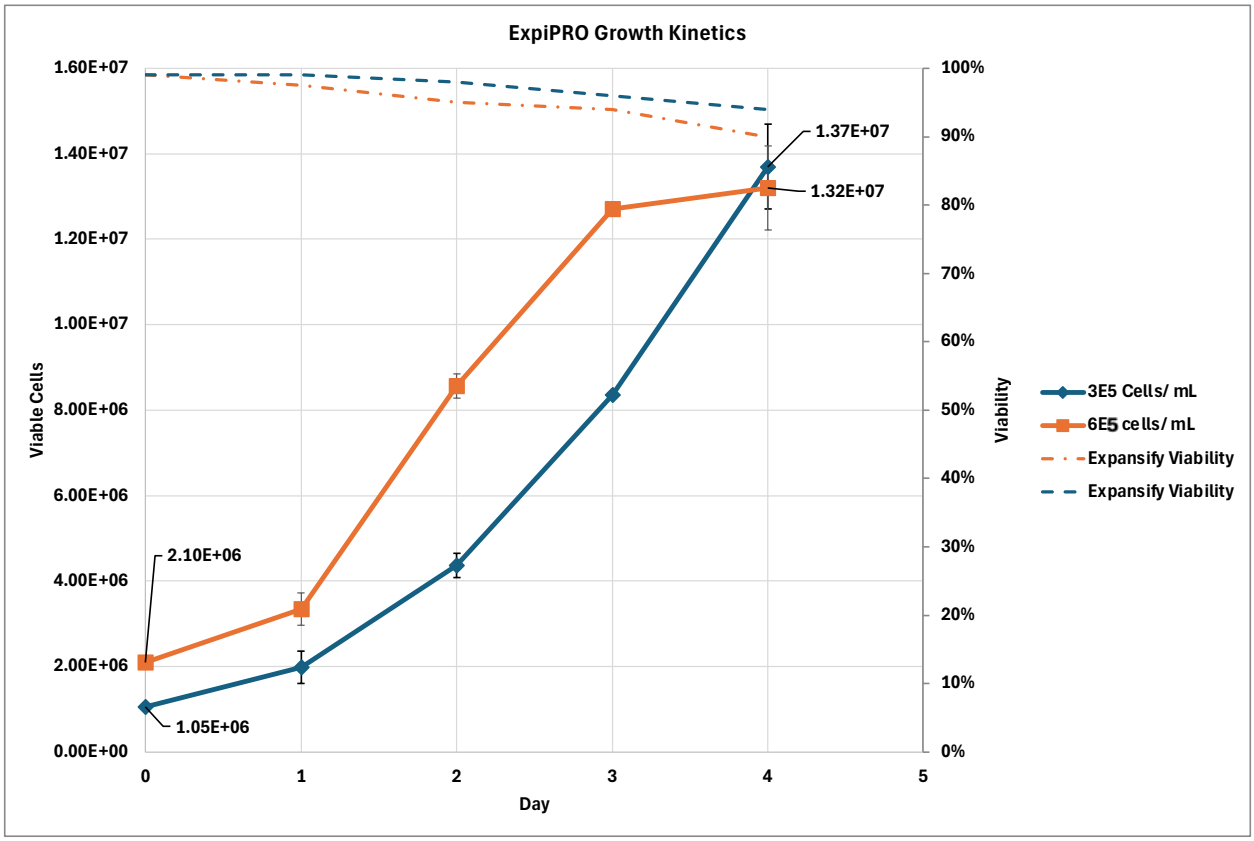
Purpose: Evaluate the impact of initial seeding density on growth kinetics and carrying capacity of Expi293PRO cells cultured in Expansify™ 24-well plates under static media conditions.

Experimental set-up:

- D0: seed 3×10^5 or 6×10^5 cells/ ml (3.5 mL total) into wells.
- Static culture, humidified incubator, 8% CO_2.
- Media: Expi293PRO Expression (media not changed over the course of this study).
- Duplicate wells counted and averaged for each timepoint.

Results:

- Peak cell harvest average 1.35×10^7 total cells/ well for both conditions, regardless of initial seeding conditions.
- Viability >90% over duration of culture.



CHO-K1 (Suspension Cell)

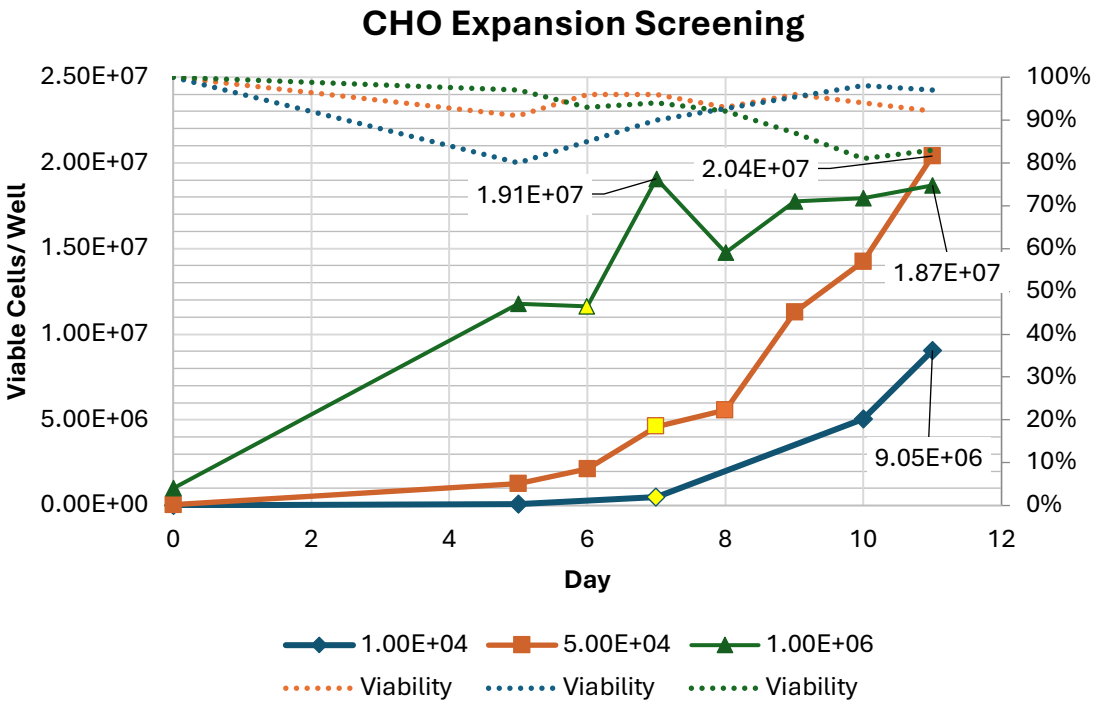
Purpose: Evaluate growth kinetics and cellular behavior of CHO suspension cells across a range of seeding densities in Expansify™ 24-well plates.

Experimental set up:

- Seed 1×10^4, 5×10^4 ,1×10^6 CHO cells/ well + 3.5 mL media.
- Media: EX-CELL CD CHO Fusion.
- Incubator: humidified, 5% CO_2, static culture.
- Complete 3.5 mL media exchange was performed on a single designated day (highlighted in yellow) to mitigate L-glutamine degradation during the extended culture period.

Results:

- CHO cells cultured in the Expansify™ 24-well plate demonstrated robust expansion capabilities from a wide range of initial seeding conditions following a 11-day culture.
    - 1×10^4 initial seeding density: 905-fold expansion
    - 5×10^4 initial seeding density: 408-fold expansion
    - 1×10^6 initial seeding density: 19-fold expansion



NISTCHO (CHO Suspension Cell)

Purpose: Assess the impact of culture device format on cell behavior and monoclonal antibody production characteristics of the NISTCHO cNISTmAb producer cell line (NIST Catalog No. RM 8675), using Expansify™ 24-well plates and traditional 125 mL shake flasks.

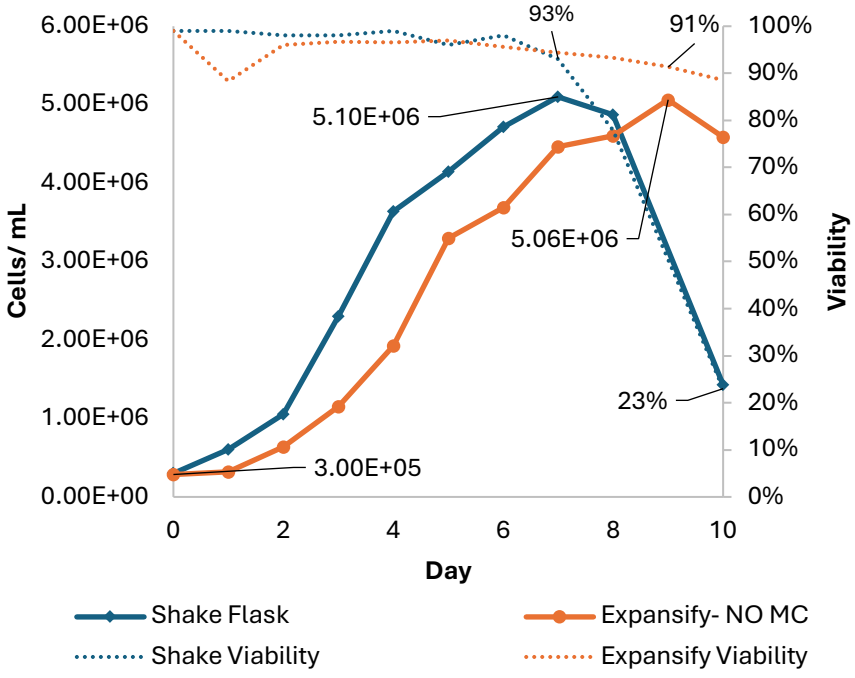
Experimental set up:

- 125 mL shake flask and Expansify™ 24- well seeded at 3×10^5 cells/ mL.
  - Shake flask: 125 mL shake flask, 30 mL fill, 5% CO_2, 19 mm throw, 145 RPM.
  - Expansify™ 24-well: 3.5 mL fill, 5% CO_2, static culture.
- Media: EX-CELL CD CHO Fusion

Results:

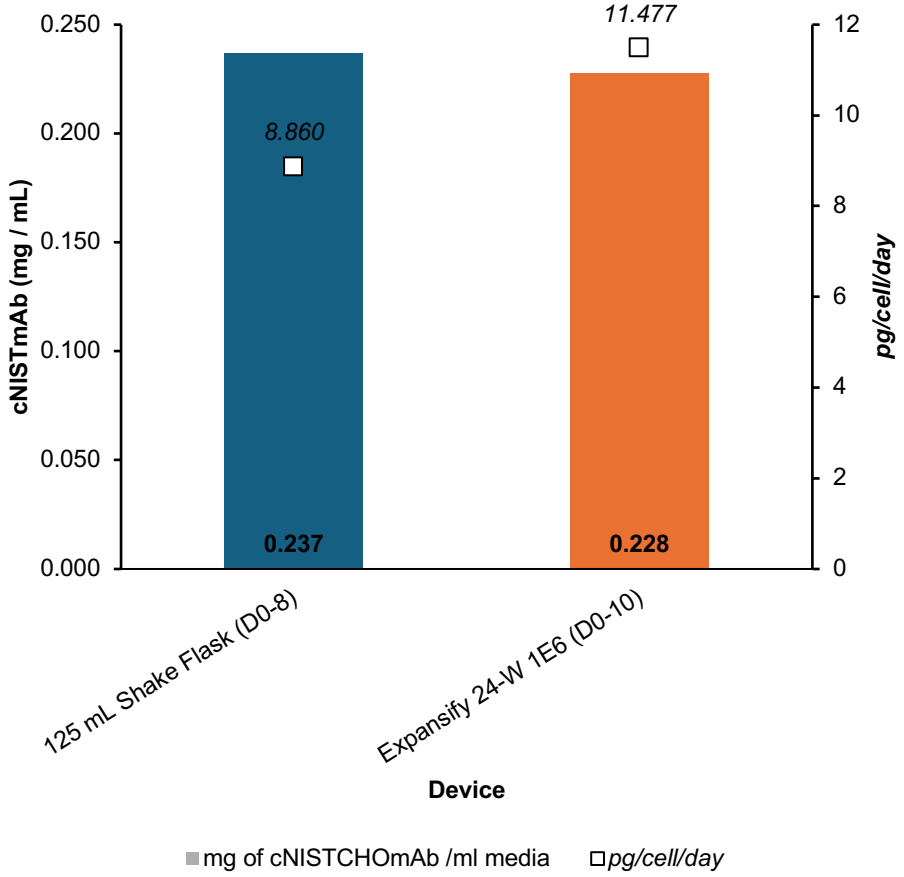
- **Figure 1 growth characteristics**: NISTCHO cells grown in shake flasks reached their peak cell density of 5.1×10^6 cells/ mL on D7, whereas Expansify™ cultures reached their peak cell density of 5.06×10^6 cells/ mL on D9. Shake flask culture exhibited a rapid viability decline from their 93% peak (day 7), 78% (day 8), to 23% (day 10). In contrast, Expansify™ cultures maintained 91% viability at peak cell density (day 9) and marginally declined to 89% viability (day 10).

Figure 1: NISTCHO Growth Chatacteristics



- **Figure 2 antibody production**: Monoclonal antibody (mAb) cultures were harvested at the first time point at which culture viability declined below 80%. Therefore, shake flask cultures were harvested and evaluated on day 8 and Expansify™ cultures were halted on day 10 (Figure 2). Final antibody concentrations were comparable between culture formats, 0.237 mg/mL for shake flask cultures and 0.228 mg/mL for Expansify™ 24-well cultures. In contrast, NISTCHO cells cultured in Expansify™ exhibited higher cell-specific productivity (11.477 pg/cell/day) relative to shake flask cultures (8.86 pg/cell/day), corresponding to a 22% increase in productivity for the same producer cell line.

Figure 2: NISTCHO Device Performance



cNISTmAb Quality Assessment

Performed by Cambridge Antibody Labs

The experimental setup above was repeated and scaled to a 500 mL shake flask (100 mL working volume) and an Expansify™ 1-well plate (100 mL working volume), both seeded at identical cell densities (3×10^5 cells/mL). Antibody production proceeded until culture viability decreased to ≤80%, at which point cultures were harvested, processed, and supernatant stored at −80°C for subsequent characterization against a purified, pre-characterized NIST reference antibody. This summary report (full report available upon request) summarizes comparative analyses of production yield, purity and aggregation, structural integrity, impurity profile, glycosylation, and binding kinetics (SPR) to the RSV target antigen.

Production & Yield

Description	Protein Concentration (mg/mL)	Volume (mL)	Protein Amount (mg)	Endotoxin by LAL assay	Buffer	Storage
500ml shake flask	0.90	94	84.6	<0.05 EU/mL	Tris-acetate, neutral pH	-80°C until use
1-Well plate	1.04	88	91.52	<0.05 EU/mL	Tris-acetate, neutral pH	-80°C until use

Purity & Aggregation

Both candidates exhibit high purity across SDS-PAGE (reducing and non-reducing) , analytical SEC, and CE-SDS. However, Expansify™ production yielded slightly lower high-molecular-weight species (aggregation), likely indicating a cleaner product profile.

Structural Integrity (Differential Scanning Fluorimetry)

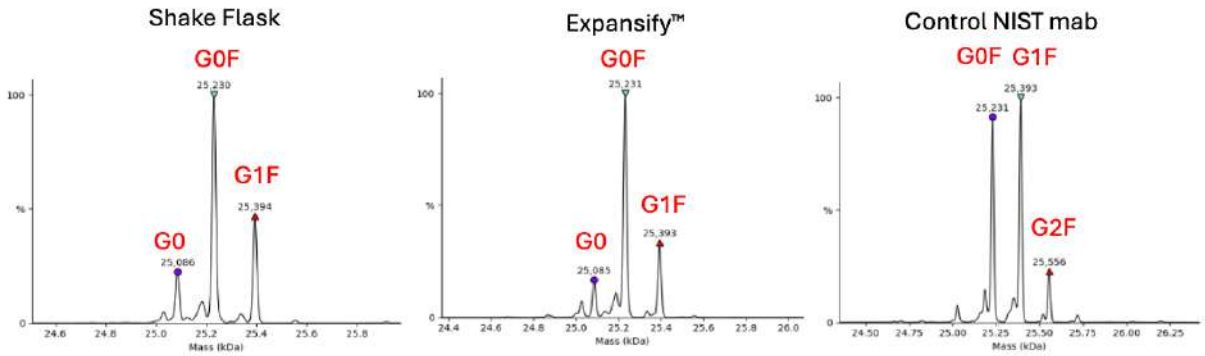
Shake flask and Expansify™ produced similar Tm values; however, both were ~1–1.5 °C lower than the control NIST mAb.

Impurity Profile

Both production methods contained similar levels of CHO contaminating host cell proteins. However, Expansify™ produced mAb contained ~3x lower host cell DNA (0.094 ng/mg) than its shake flask produced comparator.

Glycosylation

Shake flask and Expansify™ productions methods display highly similar glycan profiles between each other and the cNIST mAb control. There does exist a difference seen on the G0F glycan between shake flask/ XDemics produced mAb and the NIST control



Binding Affinity

Shake flask, Expansify™ , and NIST control samples all demonstrate similar binding kinetics to their target RSV antigen.

Sample	Ka (1/Ms)	Kd (1/s)	KD (M)
NIST mab	$1.21E^{06}$	$<1.0E^{-05}$	$<8.3E^{-12}$
Shake flask	$3.28E^{06}$	$<1.0E^{-05}$	$<3.0E^{-12}$
Expansify™	$1.60E^{06}$	$<1.0E^{-05}$	$<6.3E^{-12}$

HEK293T

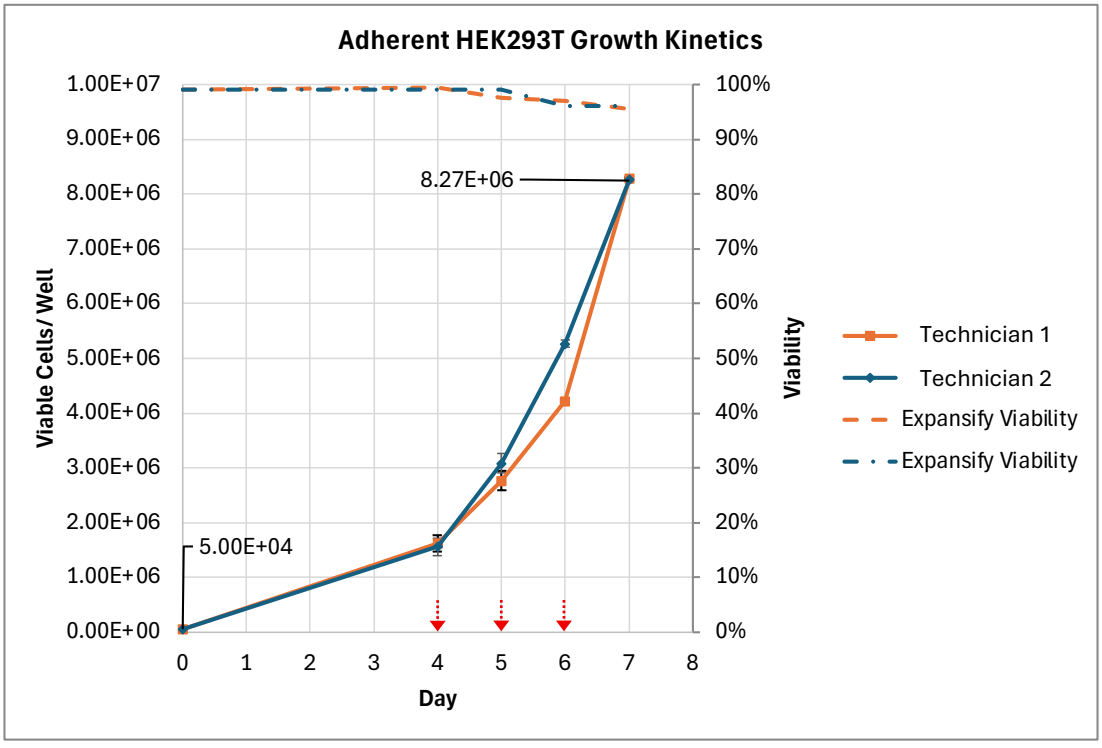
Purpose: Evaluate reproducibility of HEK293T expansion between two independent operators in Expansify™ 24-Well plates.

Experimental set up:

- Uncoated Expansify™ plate.
- 5×10^4 cells + 4.5 mL media/ well.
- Media: DMEM High Glucose + 10% FBS.
- Cultures grown in static conditions at 37C, humidified, 5% CO_2
- Duplicate wells counted and averaged at each timepoint per operator.
- Full 4.5 mL media exchange performed on days 4,5, and 6 (red dotted arrow lines).

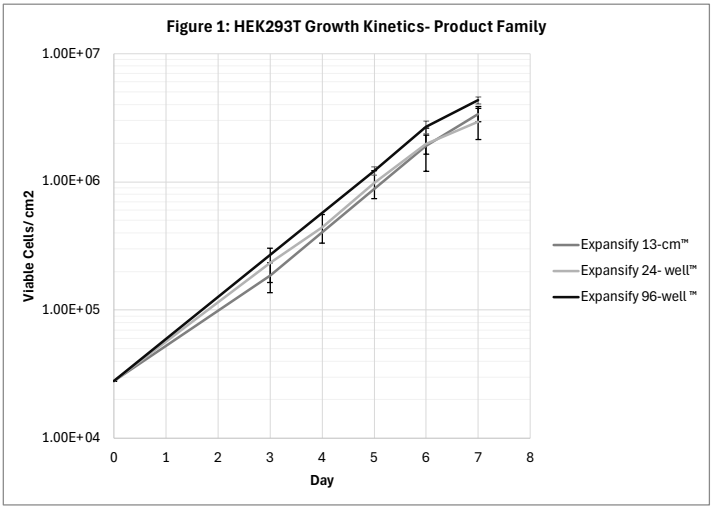
Results:

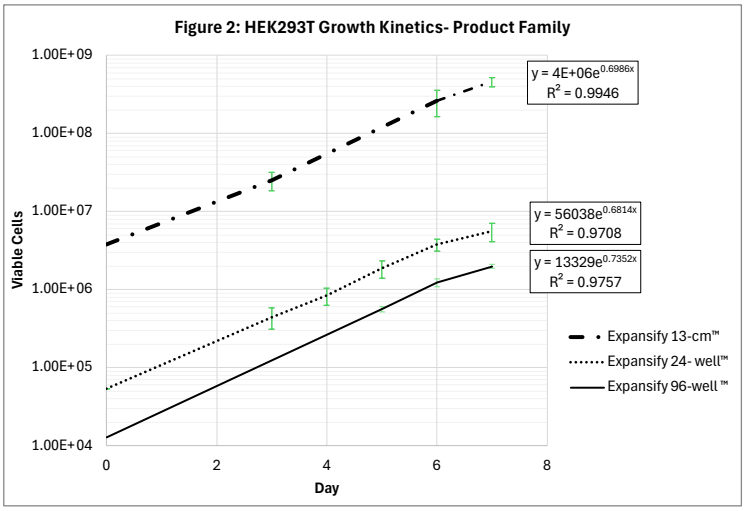
- HEK293T cells grown in Expansify™ 24-well plates exhibit a high degree of reproducibility between independent operators.
    - Average doubling time: 22.48 ± 0.32 hours
        - Trial 1 average doubling time: 22.7 hours
        - Trial 2 average doubling time: 22.25 hours
    - Viability > 95% at all timepoints (Trypan exclusion cell counting).
    - Percent difference between growth curves = 3.2%

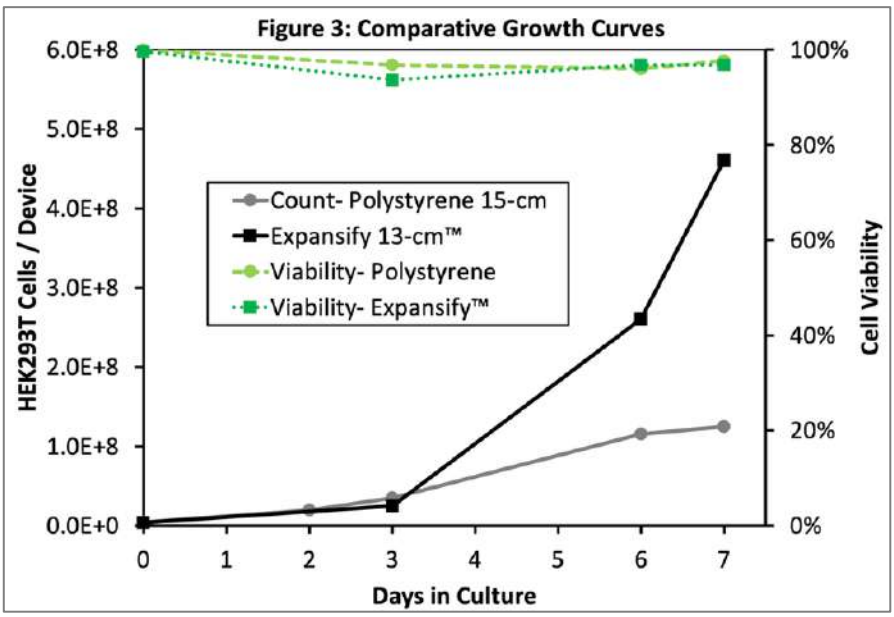


Scalability with Expansify™ (HEK293T)

Culturing with Expansify™ enables scalable cell culture applications while maintaining consistent cell growth behavior across an approximately 300-fold increase in culture scale up. Data presented in Figures 1 and 2 demonstrate reproducible growth kinetics across multiple culture formats. Specifically, HEK293T cells cultured in uncoated ECM Expansify™ *beta* 96-well plates (0.46 cm^2) exhibited growth profiles comparable to those observed in 24-well plates (2 cm^2) and 13 cm dishes (136 cm^2). All culture conditions were evaluated in triplicate and assessed across the Expansify™ product portfolio. Across all formats, HEK293T cells demonstrated a mean doubling time of 23.59 ± 0.92 hours (SD), indicating robust scalability and platform consistency. Additionally, Figure 3 illustrates sustained exponential growth of HEK cells cultured in the Expansify™ platform relative to conventional 15-cm polystyrene Petri dishes.

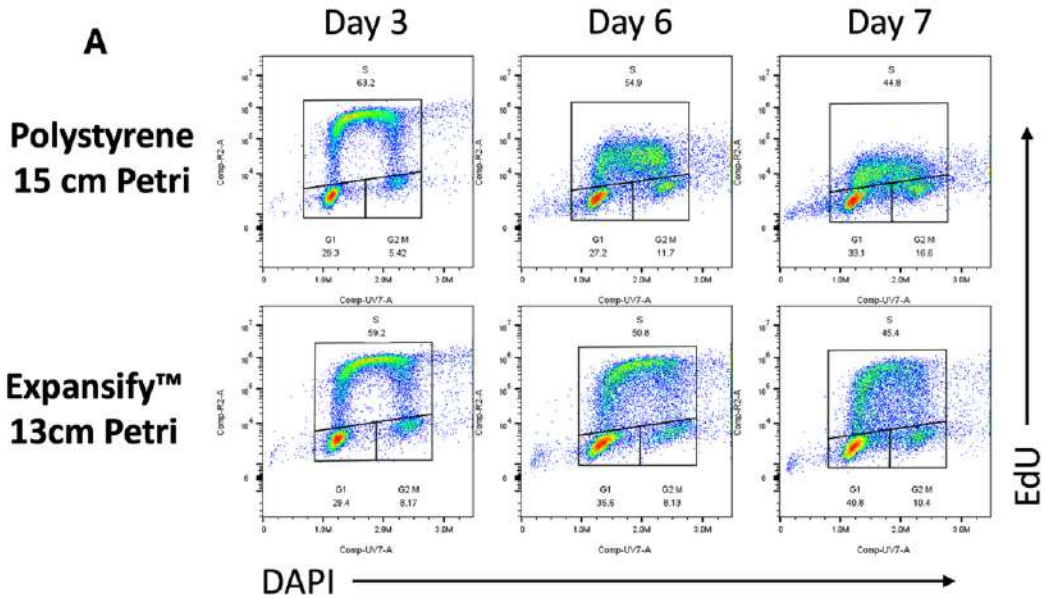




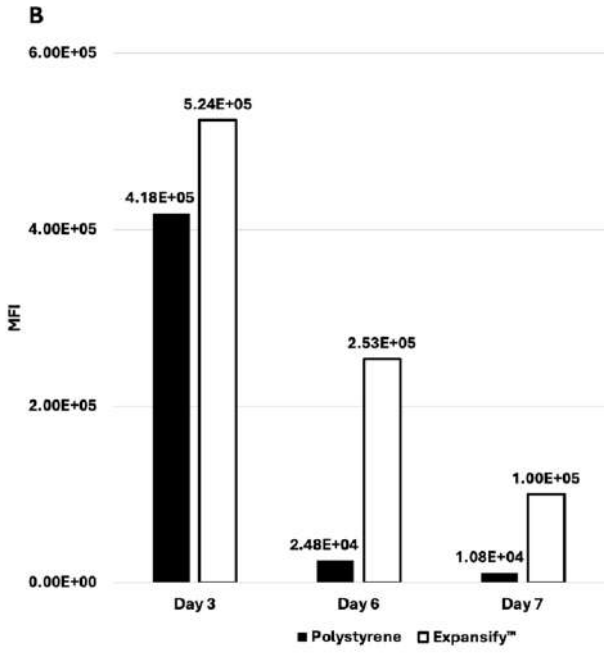


EdU- HEK293T Grown in Expansify™ or Polystyrene Culture Ware

A: EdU staining for cell cycle state of adherent HEK293T cells expanded in PS petri versus uncoated ECM Expansify™ 13cm petri (previous form factor). After reaching 2D confluence on Day 3, cells in the PS dishes continued to overgrow and demonstrated a diminished S-phase state. Cells in the Expansify™ dish maintained an appreciable S-phase state up to near 3D confluence.



B: Quantitation of relative rate of DNA synthesis for cells in S-phase across PS and Expansify™ dishes.



HeLa- GFP (Adherent cell line)

Purpose: Characterize the average growth kinetics (n = 5) of HeLa-GFP cultures in Expansify™ 24-well plates and to assess whether prolonged, exclusive passaging in Expansify™ plates results in measurable changes in cell expansion kinetics.

Experimental set-up:

- D0: introduce 5.3×10^4 total cells per well + 4.5 mL of media introduced to wells.
    - Full 4.5 mL media exchanges occurred on days 5-7 (red arrows).
    - 4.5 mL initial fill + 3 x media exchanges= 18 mL media utilized.
- Media: DMEM High Glucose+ 10% FBS.
- Incubator: humidified, 5% CO_2.
- HeLa- GFP cells harvested on day 8 washed 1x with PBS, dissociated into a single cell suspension with 0.75 mL TryplE (10- 15 min incubation + mid incubation trituration), and used to seed new wells for Expansify™ passaging.

Results:

- Cultures reliably exhibited >170 x fold expansion within a single well (Figure 1).
- Replicate cultures (n=5) consistently demonstrate 26.29 hrs ± 1.35 hrs (SD) doubling time (Figure 2).
- HeLa-GFP cultures demonstrated reproducible growth characteristics across 5 serial passages, capable of repeated low to high density passages over 43 days of continuous culture in Expansify™ (Figure 3).

Figure 1: Average HeLa-GFP Growth Kinetics in HDCR 24-Well

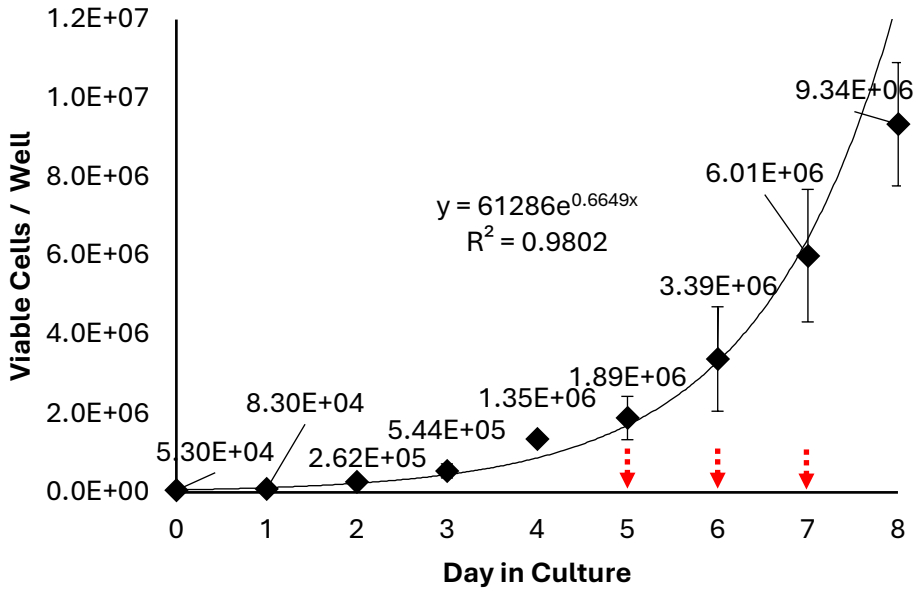


Figure 2: HeLa-GFP Serial Propagation in Expansify™ 24-Well Plate

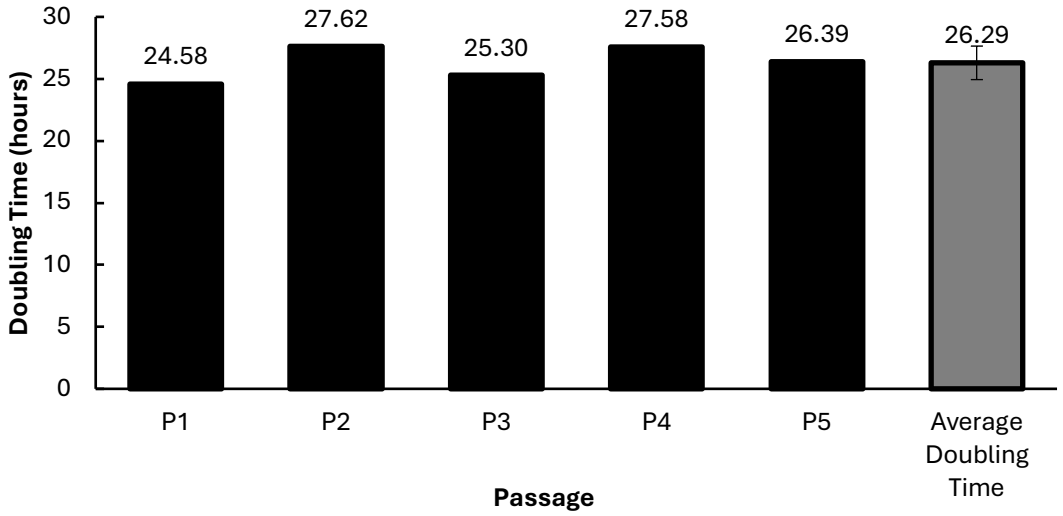
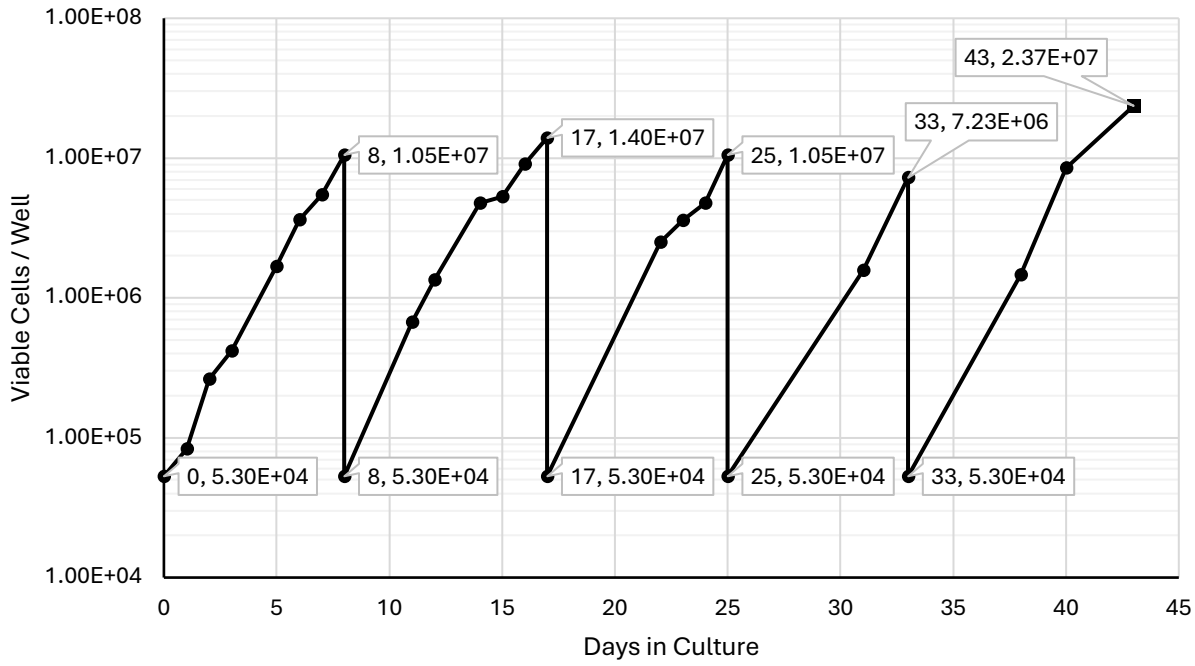


Figure 3: HeLa-GFP Serial Propagation in Expansify™ 24-Well Plate



Appendix

Vitronectin coating for adherent Expansify™ cell culture

Purpose:

Many adherent cell types exhibit substrate-specific attachment preferences mediated by extracellular matrix interactions. For applications involving silicone-based culture devices, surface coating with ECM proteins may improve cell attachment and viability. This protocol outlines an approach for culturing adherent cell lines on vitronectin-coated Expansify plates and may serve as a starting point for adapting adherent cell culture systems onto silicone platforms, if necessary.

Materials:

- Recombinant Vitronectin, Truncated (e.g. Gibco™ A400457)
- Expansify cultureware
- Pasteur Pipette (e.g. CELLTREAT Polystyrene Plasteur Pasteur Pipette catalog #229280)
- PBS (calcium magnesium free)
- Centrifuge with well plate rotor

Procedure:

1. Dilute vitronectin with PBS to generate working concentration.
    a. 2-5ug/ mL for most immortalized cell lines.
    b. 5-10 ug/ mL for most stem cell work.
    c. Experimentally determine optimal coating concentration for best results.
2. Introduce 1 mL of coating solution to each well of a 24 well plate or 35 mL for 1-Well plates.
3. Counterbalance with deep well plate and centrifuge plate at 200 x g for 1 min.
4. Remove plates from centrifuge.
5. (Recommended) Wrap plate with clean parafilm to seal plate and minimize evaporation. Place in 4°C for a minimum of 24 hrs and up to 72 hrs for protein adsorption to silicone.
    a. (*Alternative*) Plates may be incubated at RT for ≥ 4 hrs for immortalized cell lines or ≥ 6 hrs for stem cells.
6. Remove plate from 4°C and bring to room temperature (at least ~30 min).
7. Aspirate using the 0.25 mL SureShelf™.
8. Wash with full well volume of PBS or media, then aspirate using the 0.25 mL SureShelf™.
9. The plate is now ready for cell culture.

Scaling between Expansify™ device family

The Expansify product family is designed with true linear scalability in mind. Every format from the 96-Well to the GigaCell shares a common architecture, and scalability across the portfolio is governed by membrane surface area. This means that once a process is established at any format, it can be translated directly to any other format without optimization, simply by applying a surface area–based scaling factor.

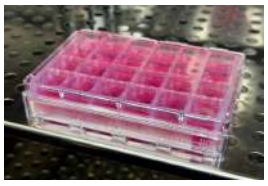
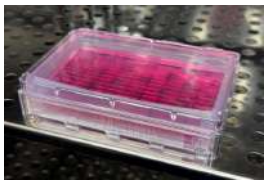
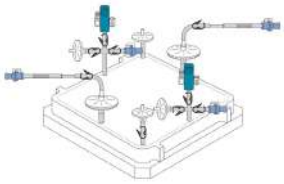
How to Calculate Your Scaling Factor

Each format in the Expansify portfolio has a defined growth surface area (cm^2), listed in the Product Attributes table below. To scale between any two formats, divide the target format's surface area by the source format's surface area:

Scaling Factor = Target Surface Area (cm^2) ÷ Source Surface Area (cm^2)

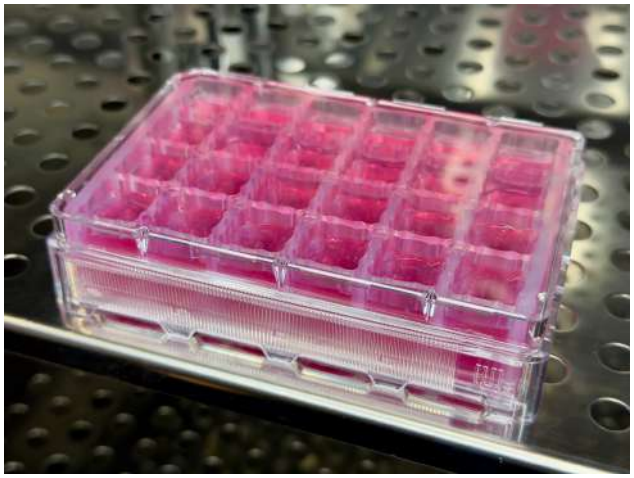
All process inputs and outputs are scale by this factor, including:

- Cell seeding density and total cell input
- Total media consumed (Media volume and feed volumes)
- Enzyme volume (e.g., for dissociation or harvest)
- Expected cell or product output

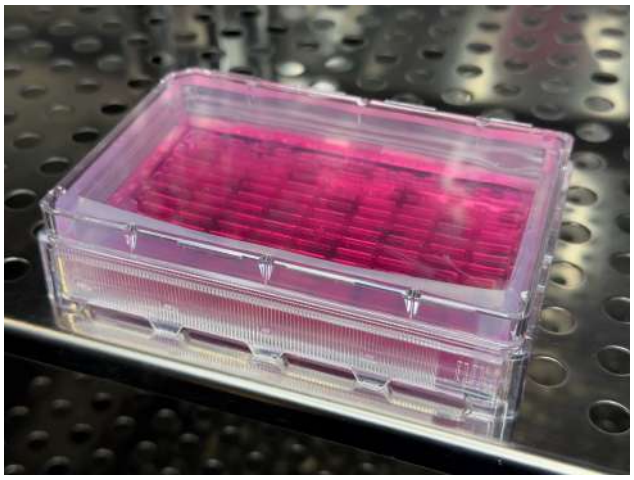
Style	96-Well	24-Well	1-Well	GigaCell™
Product				
Cell Yield (e.g. HEK293T)	3 million/ well	20 million/ well	500 million	>3.5 Billion
Surface Area	0.32 cm^2/ well	2 cm^2/ well	70 cm^2	388 cm^2
Niche Volume	10 µL	80 µL	2.7 mL	14.6 mL
Max Fill Volume	0.55 mL	4.5 mL	100 mL	1.8 L
Features	• Cell line development • High • Imageable • Centrifuge compatible • Standard ANSI/ SLAS footprint • Automation ready	• Process development • Imageable • Centrifuge compatible • Standard ANSI/ SLAS footprint • Automation ready	• Production scale up • Imageable • Centrifuge compatible • Standard ANSI/ SLAS footprint • Automation ready	• Production scale up • Closed system • Imageable • cGMP compliant (Q1 2027)

Resources

Technical Help: support@xdemics.com



Expansify™ 24-Well plate on incubator shelf, filled with 4.5 mL of media.



Expansify™ 1-Well plate on incubator shelf, filled with 100 mL of media.

Check out our full 24-Well instruction series on YouTube + keep up with our latest discoveries & talks!