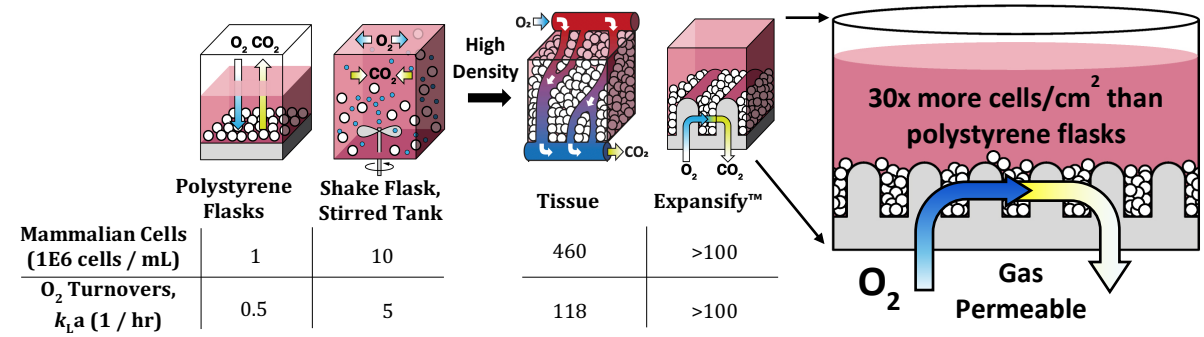
[U] XDemics

Expanding possibilities

Introduction

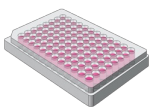
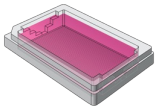
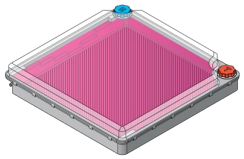
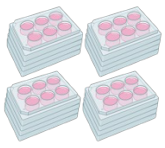
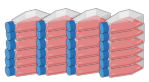
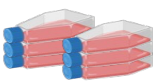
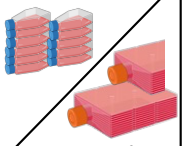
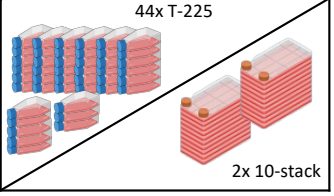
Expansify™ respiring cultureware features a series of gas-permeable ridges and grooves engineered to enhance oxygen transfer, supporting the intensified culture of adherent, spheroid, and suspension cell types. The platform's proprietary architecture is derived from a repeating unit-cell geometry (Figure 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	5	118	>100

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

Yield	96-Well Launch 2026	24-Well Available Now	6-Well Launch 2027	Single-Well Available Now	Gigacell™ Tray Launch 2026 (Beta Units H1)
1 cm² Expansify™ yields ~ 3-6 x 10^6 cells					
Plates / Incubator	300	300	300	300	20
Surface area (cm²)	0.45	2	10	70	390
Total Cells (e.g. HEK293T)	1 million per well	8 million per well	35 million per well	250 million per plate	>1 billion per tray
1 cm² Polystyrene yields ~ 1e5 cells	 16 x 6-well plates	 24 x T-75	 6 x T-225	11 x T-225  1.5 x 1720 cm² flask	44x T-225  2x 10-stack

Contents

K562 (Suspension cell line)

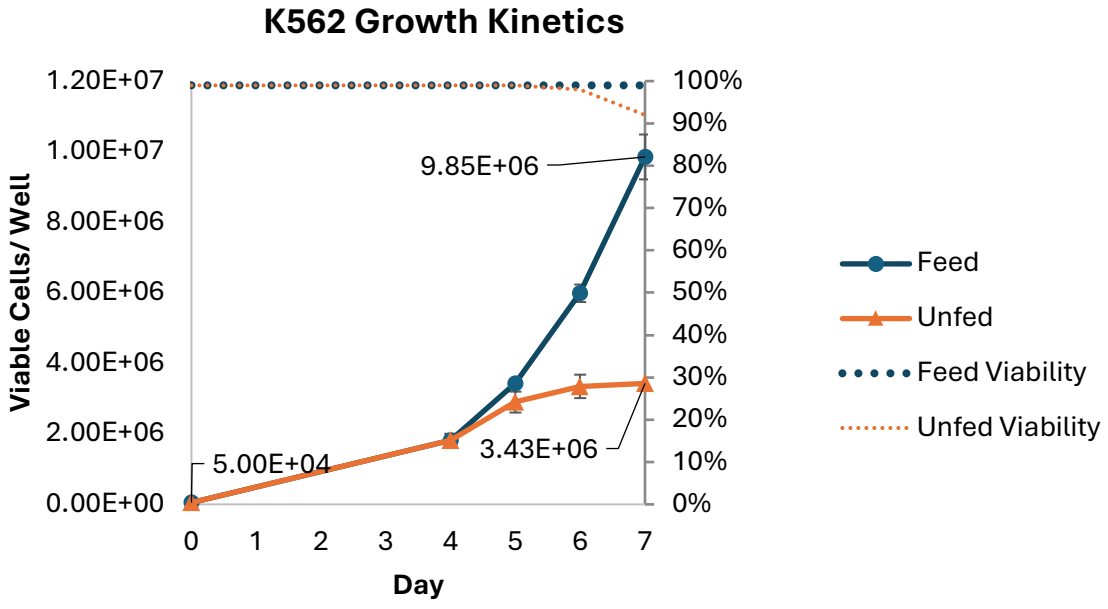
Purpose: Evaluate growth kinetics of fed vs unfed suspension K562 cells in Expansify™ 24-well plates over one week.

Experimental set-up:

- D0: introduce 5×10^4 total cells per well on D0.
    - 3.5 mL of total media introduced to wells on D0.
        - Blue fed cohort received full media exchanges (3.5 mL) on days 4-6.
        - Orange unfed cohort grew in the initial 3.5 mL of media on D0 and received no further media exchanges over the duration of the experiment.
- Media: RPMI 1640 + 10% FBS.
- Duplicate wells counted 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.
    - 14 mL of media used over duration of culture (3.5 mL initial fill on D0 + 3.5 mL media exchanges on days 4,5, and 6).
- 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.



Expi293PRO (Suspension HEK cell line)

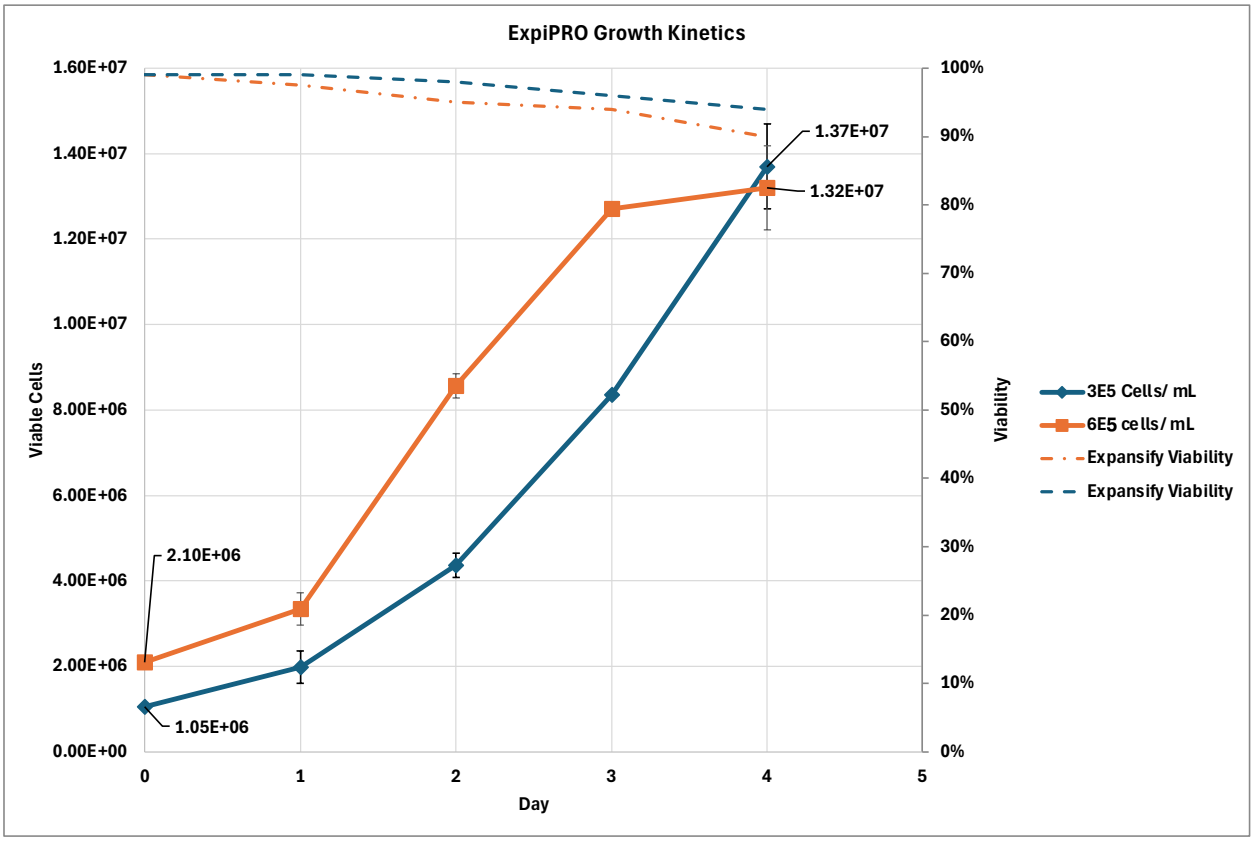
Purpose: Evaluate the impact of initial seeding density on growth kinetics and carrying capacity of Expi293PRO cells cultured in Expansify™ 24-well plates under static media conditions.

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 (Suspension Cell)

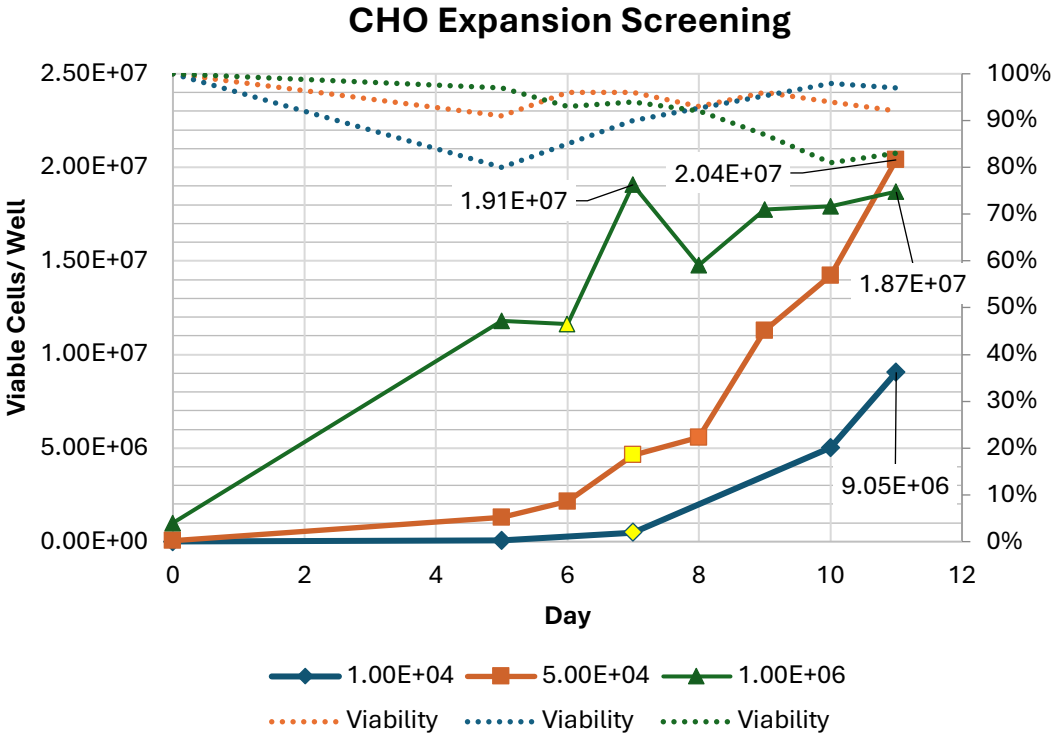
Purpose: Evaluate growth kinetics and cellular behavior of CHO suspension cells across a range of seeding densities in Expansify™ 24-well plates.

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 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 1: NISTCHO Growth Chatacteristics

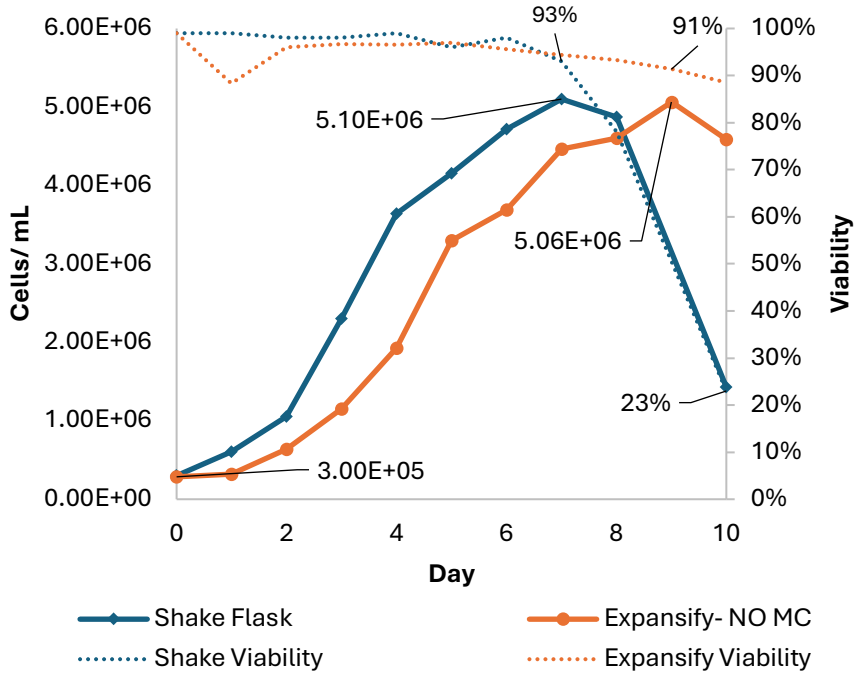
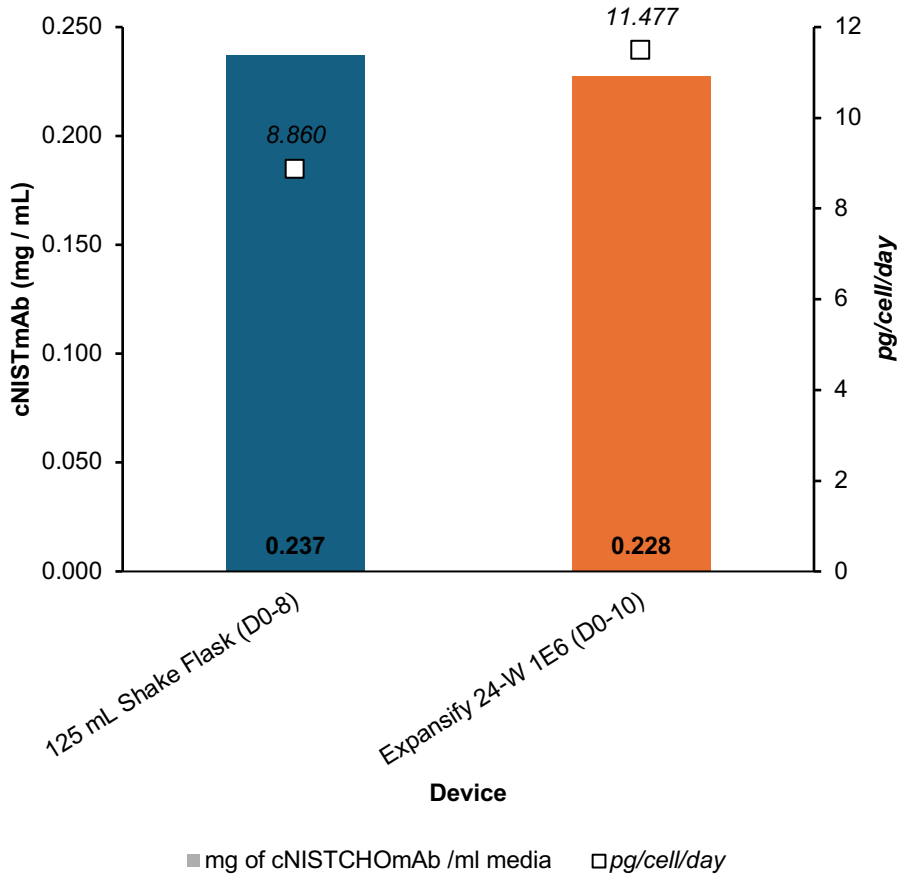


Figure 2: NISTCHO Device Performance



Adherent HEK293T

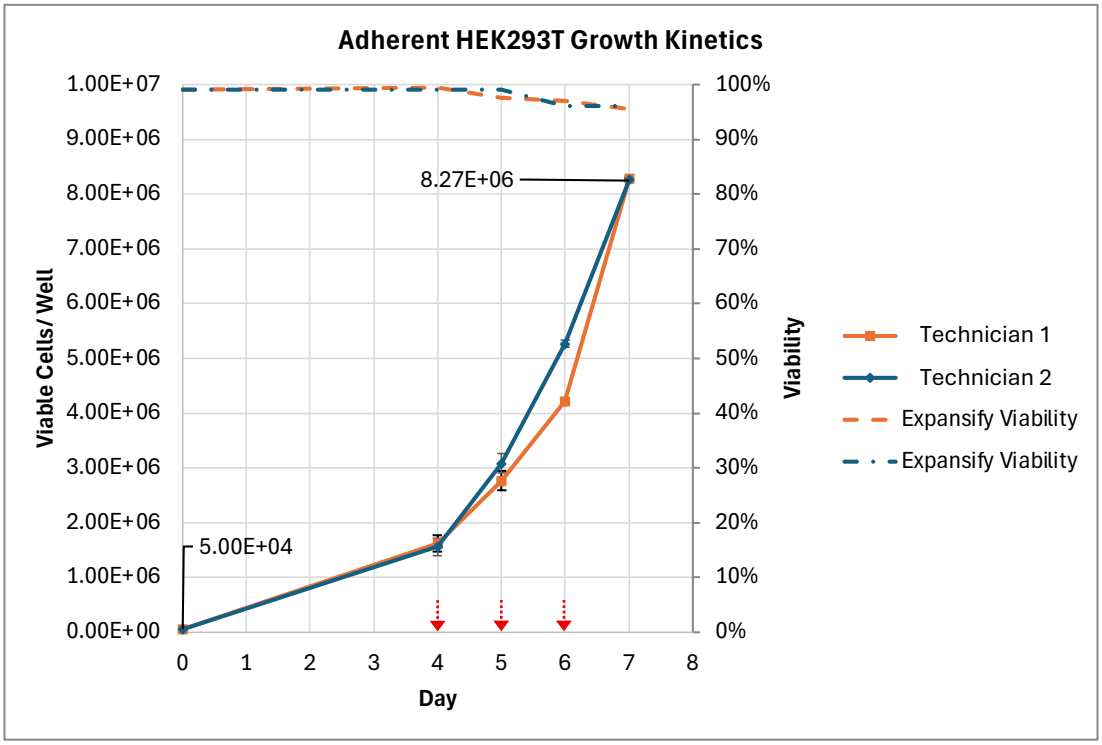
Purpose: Evaluate reproducibility of HEK293T expansion between two independent operators in Expansify™ 24-Well plates.

Experimental set up:

- 5×10^4 cells + 3.5 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 3.5 mL media exchange performed on days 4,5, and 6 (red dotted arrow lines).

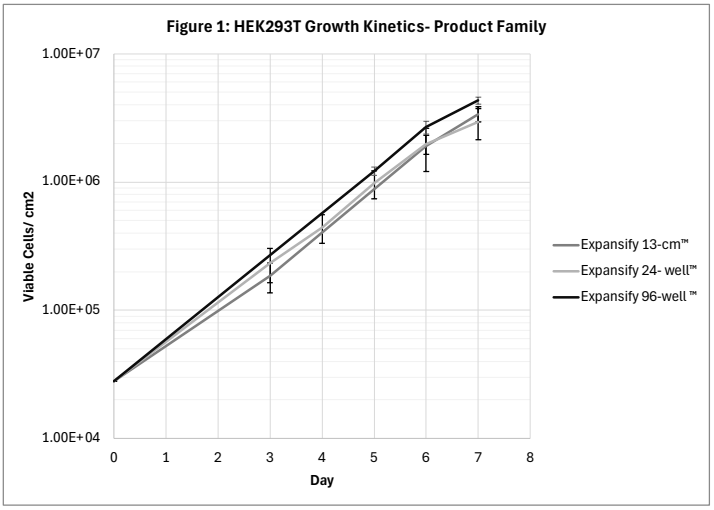
Results:

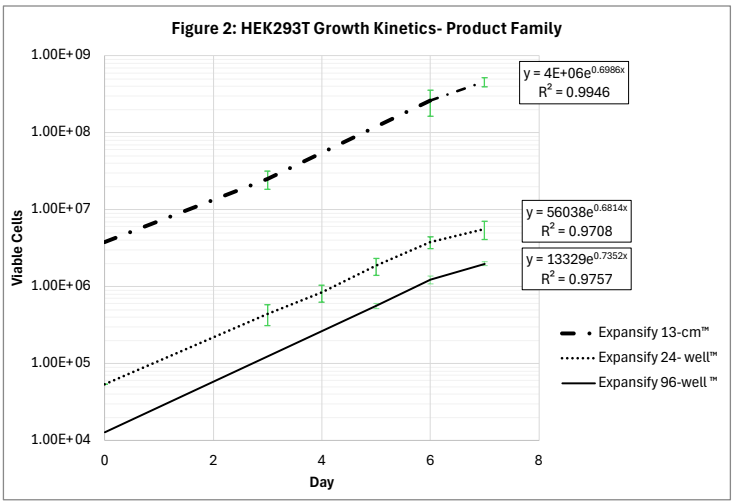
- HEK293T cells grown in Expansify™ 24-well plates exhibit a high degree of reproducibility between independent operators.
    - Average doubling time: 22.48 ± 0.32 hours
        - Trial 1 average doubling time: 22.7 hours
        - Trial 2 average doubling time: 22.25 hours
    - Viability > 95% at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

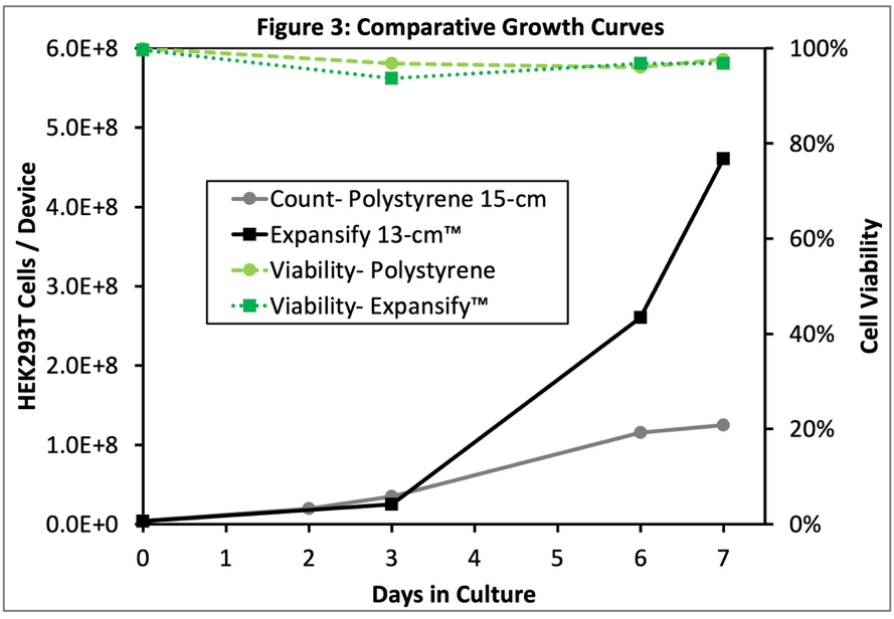


Scalability with Expansify™ (HEK293T)

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 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.

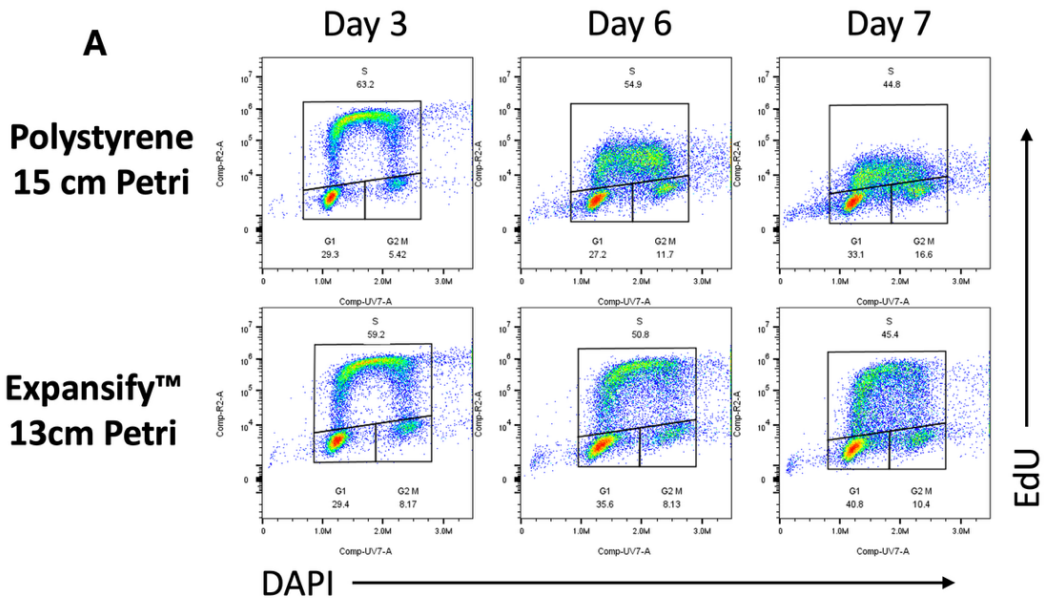




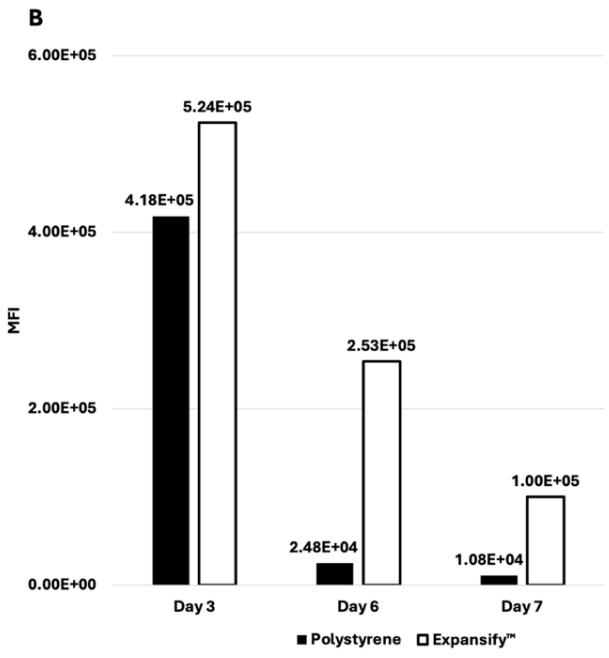


EdU- HEK293T Grown in Expansify™ or Polystyrene Culture Ware

A: EdU staining for cell cycle state of adherent HEK293T cells expanded in PS petri versus Expansify™ 13cm petri. 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 + 3.5 mL of media introduced to wells.
    - Full 3.5 mL media exchanges occurred on days 5-7 (red arrows).
    - 3.5 mL initial fill + 3 x media exchanges= 14 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

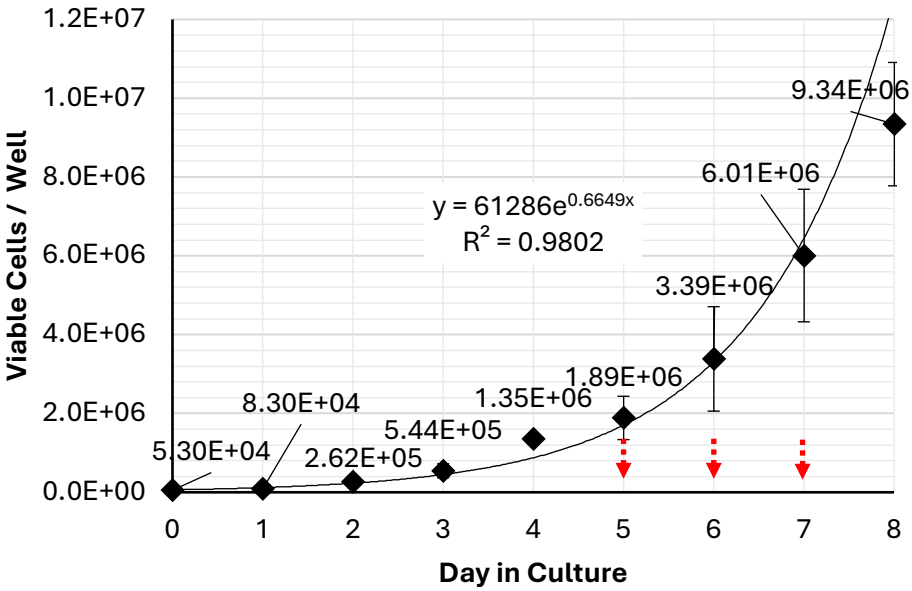


Figure 2: HeLa-GFP Serial Propagation in Expansfy 24-Well Plate

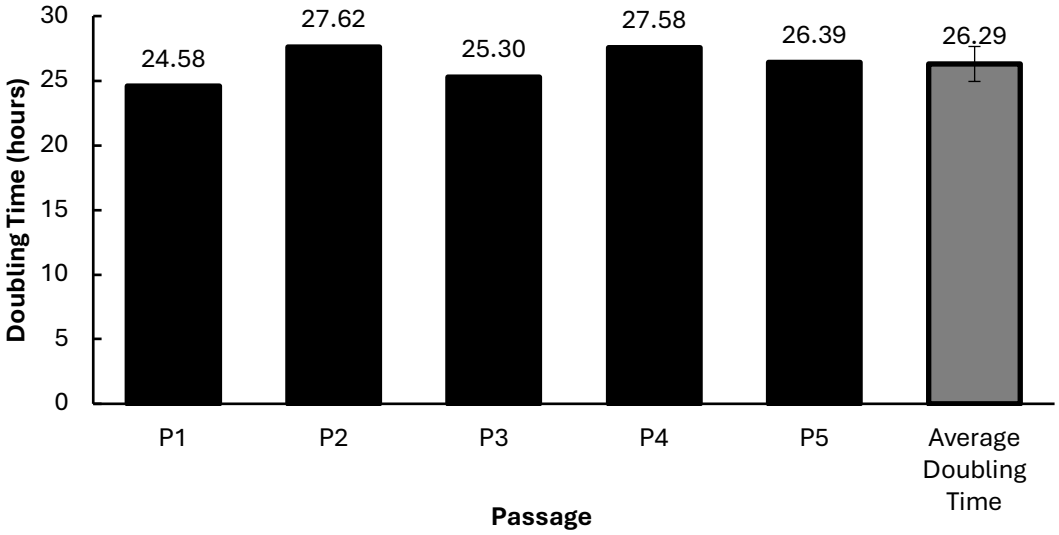


Figure 3: HeLa-GFP Serial Propagation in Expansify™ 24-Well Plate