[][] XDemics

Expansify™ Cell Culture Methods- K562 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 K562 cells in an Expansify™ 24-Well Plate. 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, including both a standard feeding schedule and an unfed condition to evaluate cell growth in the absence of media exchange. 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: K562 (ATCC® CCL-243™).
- Single-use glass or polystyrene Pasteur pipettes are required for fluid handling (e.g., CELLTREAT® Polystyrene Pasteur Pipette, Cat. No. 229280).
- Culture medium: RPMI 1640 (Gibco, Cat. No. 11-875-093) supplemented with 10% Fisherbrand™ Research Grade Fetal Bovine Serum, Canadian Sourced (Cat. No. FB12999102).
- Wide-bore P1000 pipette tip (recommended)
  NOTE: Equivalent products from other manufacturers may be substituted for the culture medium, FBS 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

Day 0: Culture Initiation

1. Add 1 mL of complete culture medium to the desired wells of the Expansify™ 24-Well Plate.
2. Centrifuge the plate at 200 × g for 1 minute. Use an appropriate Expansify™ plate or deep-well plate as a balance. This step is required before proceeding to the next step.
3. Seed K562 cells at 2.5 × 10^4 cells/cm^2 into the desired wells of the Expansify™ 24-Well Plate.
4. Adjust the final culture volume to 4.5 mL per well.
5. Following seeding, gently homogenize the cell suspension to promote uniform cell distribution and settling throughout the culture niches.
6. Monitor and image cultures as needed throughout the culture period (e.g., Days 1–7).

Days 4, 5 and 6: Medium Exchange

Two culture arms were evaluated over the course of the experiment:

1. Fed arm: Perform complete medium exchanges on Days 4, 5, and 6:
    a. 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™.
2. Unfed arm: Cultures were maintained in the initial 4.5 mL of medium added on Day 0, with no subsequent medium exchanges for the duration of the experiment.

Day 7: Cell Harvest

1. Harvest cells from the Expansify™ 24-Well Plate:
    a. 24-Well Plate (Day 7):
        i. **Remove spent medium:** Keep the plate level on the work surface. Using a Pasteur pipette, aspirate the spent medium to a known SureShelves™ level, for example the 1 mL SureShelves™, so that the residual volume in the well is known.
        ii. **Resuspend the cells:** Gently mix the culture to resuspend the cells and achieve a uniform single-cell suspension.
        iii. **Collect the cells:** Collect the resulting cell suspension for downstream processing, analysis, or further expansion.
        iv. **Determine cell concentration and viability:** Determine cell concentration and viability using Trypan Blue staining and a Countess™ 3 Automated Cell Counter.
        NOTE: If the cell concentration exceeds the measurement range of the cell counter, dilute the cell suspension with culture medium or PBS as needed before counting.

[][] XDemics

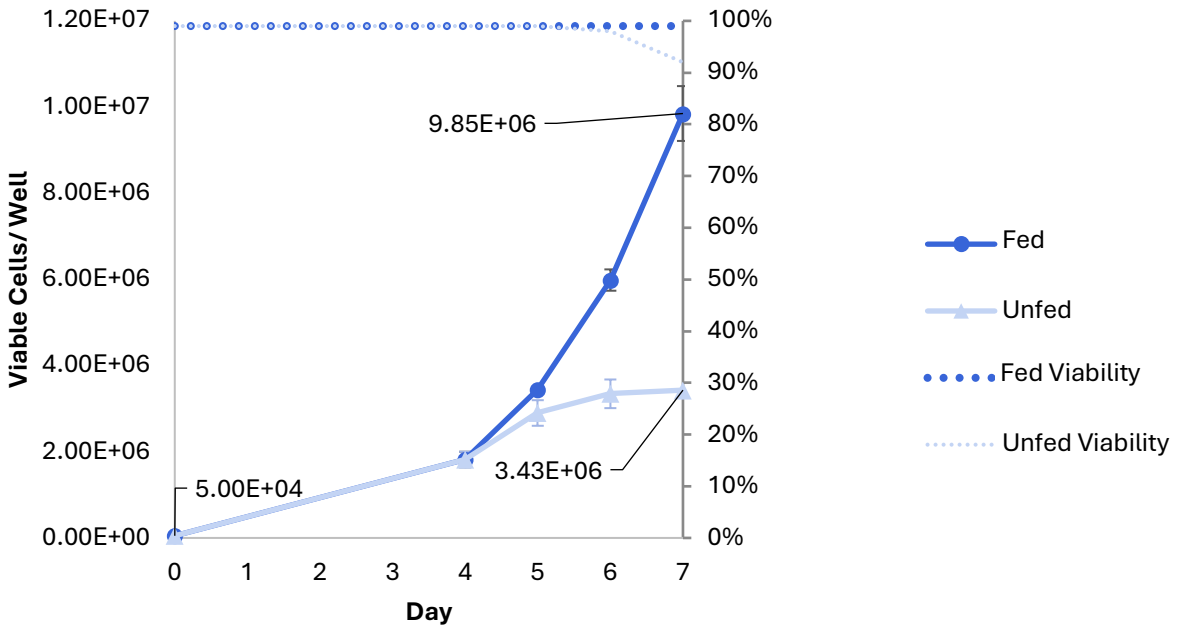
Performance Summary

K562 cells cultured in Expansify™ plates demonstrated robust expansion and high viability over the 7-day culture period. Data represent two independently harvested wells per time point (n = 2), with separate wells destructively harvested at each time point. In the fed culture condition, cells exhibited exponential growth with a mean doubling time of 20.36 hours, achieving greater than 190-fold expansion without passaging while maintaining >98% viability at all measured time points as determined by Trypan Blue exclusion.

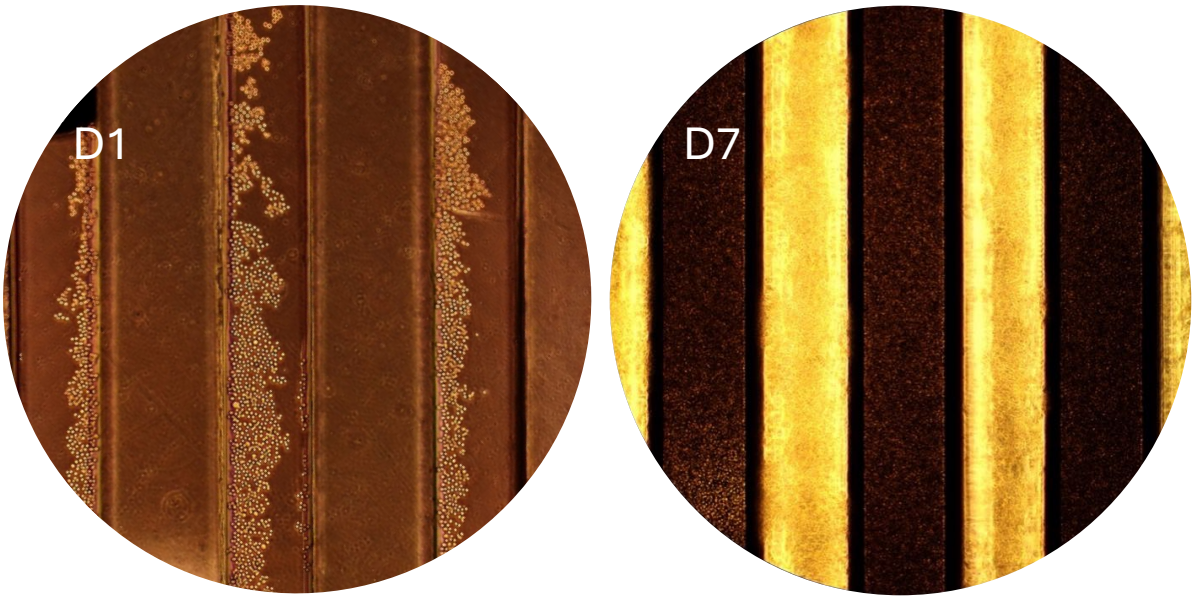
The fed culture condition reached a peak density of approximately 9.85×10^6 cells per well by Day 7. A total of 18 mL of culture medium per well was used over the duration of the experiment, consisting of an initial 4.5 mL fill volume and complete 4.5 mL medium exchanges on Days 4, 5, and 6.

In the unfed culture condition, cells displayed exponential growth through approximately Day 4 before transitioning into a plateau phase for the remainder of the experiment. Despite the absence of medium exchanges, cultures achieved approximately 68-fold expansion over 7 days while maintaining greater than 90% viability throughout the culture period.

K562 Growth Performance in Expansify™ 24-Well Plates



K562 Expansion in Expansify 24-Well Plates



Representative images of K562 cultures on Days 1 and 7. Following initial localization within the culture niches, K562 cells underwent rapid expansion and progressively populated the oxygenating surfaces over the 7-day culture period. Images were acquired using an ECHO Revolve microscope at 4× magnification.