Cell Growth Capability of Esco CelCulture® Incubator Touch Screen (CCL-170B-8-TS)

by Lulu Aulia Rahmah, Ramadhanul F. Yandra

Summary

The cell growth and viability assessment of CCL-170B-8-TS across different passages (P2, P4, and P5) demonstrated consistently high proliferation rates and stable viability above 90% over a five-day incubation period. Such stability indicates that the CCL-170B-8-TS system provides a highly controlled and supportive environment for the cultivation of HEK293T cells. Importantly, the uniformity of outcomes across multiple passages highlights the system's ability to preserve cellular integrity even after repeated subculturing. This feature is critical in laboratory and industrial settings where reproducibility, consistency, and reliability are paramount. Deviations in environmental conditions often compromise cellular performance, but the CCL-170B-8-TS ensures precision and consistency, thereby minimizing experimental variability. These results confirm the reliability and robustness of CCL-170B-8-TS as a dependable model for research and industrial applications.

Keywords: *CCL-170B-8-TS, cell viability, culture performance*

1. Introduction

Cell culture has become an indispensable tool in modern biological research, biotechnology, and biopharmaceutical manufacturing. Its success depends on the ability to replicate and maintain precise physiological conditions that allow cells to proliferate and remain viable in vitro. Among the various factors influencing cell culture performance, the incubator plays a pivotal role as the core instrument that regulates the microenvironment required for consistent growth and reproducibility.

CO_2 incubators are designed to provide stable and controlled conditions by maintaining environmental conditions for the cultivation of mammalian cells and other sensitive biological materials. These devices are adjustable heater (typically for cell incubation 37°C)[1], relative humidity (typically 85–90%)[2], and carbon dioxide (commonly maintained at 5%)[3] to support optimal cell growth and physiological stability. Slight deviations in these parameters can significantly affect cellular metabolism, proliferation, and viability, underscoring the importance of precise environmental control[4].

To validate performance, incubators must be evaluated not only for their ability to maintain stable environmental conditions but also for their effectiveness in supporting actual cell growth and cell viability. Growth curves provide insights into the proliferation capacity of cells under incubation, while viability measurements reflect the proportion of living cells that remain healthy during culture. Together, these parameters serve as critical indicators of how well an incubator can support robust and consistent cell culture outcomes.

2. Materials and Method

Mammalian cells (HEK293T) will be cultured in the **CCL-170B-8-TS** to monitor the cell count and viability for a period of 5 days (day 0, 2, 3, 4 and 5) for 1 passage.

2.1 Materials used in this experiment were:

1. HEK293T Cells (ATCC no: CRL-3216™)
2. CO2 Incubator (CCL-170B-8-TS)
3. Biosafety Cabinet
4. Centrifuge
5. Water Bath

6. NC-202 Cell counter and viability machine
7. Inverted Microscope
8. T-75 Flasks
9. DPBS
10. DMEM Media
11. Fetal Bovine Serum (FBS)
12. tryPLE™

2.2 CO_2 Incubator Preparation

1. Clean and set up the incubator chamber
2. Set temperature to 37˚C and CO_2 to 5%
3. Run the Sterilization cycle according to the instructions
4. Pour 400 ml deionized water into the water pan prior to the start of the study

2.3 HEK293T Cell Preparation

1. Prepare 10 mL of complete DMEM media (+ 10% FBS) into 15 mL centrifuge tube and pre-warm it to 37˚C
2. Retrieve cryovial from LN2 Tank.
3. Thaw and transfer the HEK293T cells from the cryovial into the pre-warmed complete DMEM media from the previous step.
4. Centrifuge the cells at 200 x g for 5 mins (Acceleration and Deceleration Levels: 9).
5. Aspirate and discard the supernatant.
6. Resuspend the cells with the remaining 1 mL of complete DMEM media.
7. Take a 0.1 uL of cells to dilute with 0.4 uL DPBS for cell count and viability assay (usually 5X dilution).
8. Perform manual cell count using hemocytometer, and compute the cell count and viability assay (refer to Section 6.4)
9. Add 15 mL of pre-warmed complete DMEM media to T75.
10. Add cells at a seeding density of 8 x 10^5 cells/T75 and cultivate them for 3 days at 37°C and 5% CO_2 for cell recovery. Cells will be deemed recovered after 2 passages or cell viability of 90% (whichever comes first)
    *Note, Cells required to have >50% cell viability on first passage to proceed with study

2.4 HEK293T Cell Seeding and Incubation

1. Aspirate the initial media and wash the HEK293T cells with 2ml of DPBS three times
2. Add 2mL of pre-warmed tryPLE™ select and leave the T75 in the BSC for 2 minutes to trypsinise the cells. Ensure the cells are lifted from the surface via pipetting
3. Add 4mL of pre-warmed complete DMEM media to inactivate the trypsin and transfer the content into a 15mL centrifuge tube
4. Centrifuge the cells at 200 x g for 5 mins (Acceleration and Deceleration Levels: 9)
5. Aspirate and discard the supernatant
6. Resuspend the cells in 2mL of complete DMEM media
7. Dilute 100uL cells with DPBS for cell count (e.g. 10X dilution) in a 1.5mL microcentrifuge tube (e.g. 100uL cells to 900uL DPBS)
8. Perform manual cell count using hemocytometer (refer to **Section 2.5**)
9. Prepare a master mix of HEK293T cell culture according to T75 flask (e.g. if we are seeding 5 x T75 flask, we will need 0.8e^6 x 5 cells).
10. Seed the cells into the T-75 flasks, ensure cells are well spread across the flask but shake and check the spread using an inverted microscope
11. Maintain a batch of HEK293T cells (5 x 10^5 cells/T75 flask) in a T-75 flasks for future experiments (if required)

12. Total of 5 x T-75 flasks were seeded for cell count and cell viability (Refer to 6.4) assay respectively (Label the flask with Cell-type, Passage number and time point).
13. Put the T-75 flasks for study in the middle of the incubator (middle tray, middle spot)
14. On day 0, 2, 3, 4 and 5 one flask will be removed from each zone for cell count and cell viability test (except for Day 0, data retrieved based on seeding cell count and viability). **Captures images of the cells under microscope before cell counting.**
15. Repeat the cell count and viability test (Step 1 – 14) for 1 more time with 1 more passage of cells (if required)
16. Record cells count and cell viability test and a graph will be plotted to tabulate the trend of these tests using the readings

2.5 Cell Count and Cell Viability Test

1. Aspirate the initial media and wash the HEK293T cells with 2ml of DPBS three times
2. Add 1mL of pre-warmed tryPLE™ select and leave the T75 in the BSC for 2 minutes to trypsinise the cells. Ensure the cells are lifted from the surface via pipetting
3. Add 2mL of pre-warmed complete DMEM media to inactivate the trypsin and transfer the content into a 15mL centrifuge tube
4. Centrifuge the cells at 200 x g for 5 mins (Acceleration and Deceleration Levels: 9)
5. Aspirate and discard the supernatant
6. Resuspend the cells in 2mL of complete DMEM media
7. Dilute a small sample of cells with DPBS for cell count if needed (e.g. 10X dilution) in a 1.5mL microcentrifuge tube
8. To operate the NC-202 Cell Counter and Viability machine, first mix the cell suspension thoroughly to ensure homogeneity. Then, load the sample by inserting the tip of the Via2-Cassette™ into the suspension and pressing the piston. Next, place the loaded cassette into the NucleoCounter® NC-202™, select the 'CEF' protocol, and press RUN.
9. After approximately 30 seconds, the cell concentration and viability of the sample are displayed.

Results and Discussion

The cell growth and viability data of CCL-170B-8-TS incubated from Passage 2 (P2), Passage 4 (P4), and Passage 5 (P5) show a clear trend of increasing cell number during the incubation period. In the cell count profile (Figure 1), all passages exhibited clear exponential growth beginning after day 2, with P4 achieving the highest cell density (approximately 6.0×10^7 cells/ml by day 5), followed by P2 and P5. These results underscore the strong proliferative potential of HEK293T cells cultured in the CCL-170B-8-TS incubator across multiple passages. The consistent upward trend in cell numbers throughout the five-day incubation period confirms that the cells adapt well to subculturing while maintaining robust expansion capacity. Notably, the reproducibility of this exponential growth pattern across P2, P4, and P5 highlights the reliability of the CCL-170B-TS culture system, minimizing variability and ensuring predictable performance in both research and industrial-scale applications.

In terms of cell viability, the values generally remained stable in the range of 93–97% throughout the 5 days of incubation (Figure 2), which indicates that the cells were in good condition during the culture period. However, a slight decline in viability was observed in P4 on day 5 (around 92%), while P2 and P5 maintained relatively higher values. This fluctuation may suggest the onset of stress in cells at later incubation stages, possibly due to nutrient depletion, waste accumulation, or passage-related physiological differences. Importantly, despite this minor reduction, the overall viability remained well above 90%, confirming that the CCL-170B-8-TS incubator provided a stable and supportive environment for HEK293T cells. The high and consistent viability across passages further underscores the robustness of the culture system, ensuring that the cells not only proliferate effectively but also retain their physiological integrity an essential factor for reproducibility in experimental studies and potential scale-up applications.

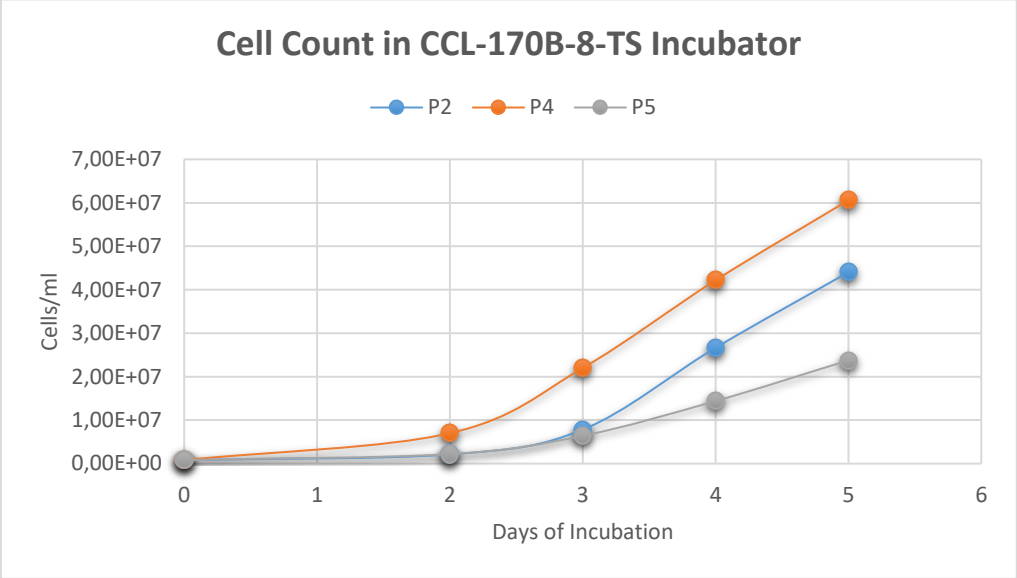


Figure 1. Cell count of HEK293T cultured in CCL-170B-8-TS incubator over 5 days of incubation at different passages (P2, P4, P5).

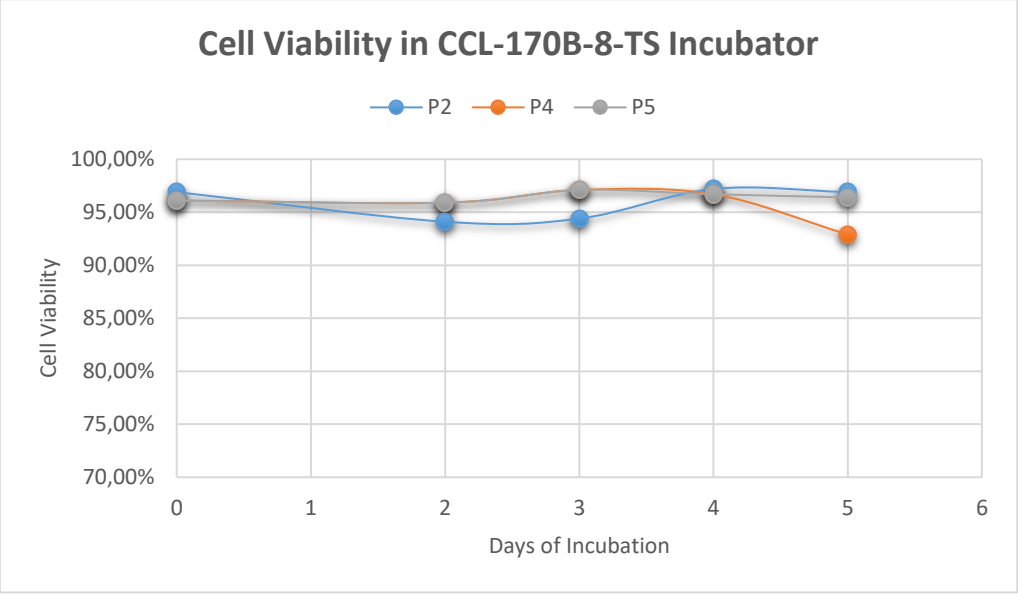
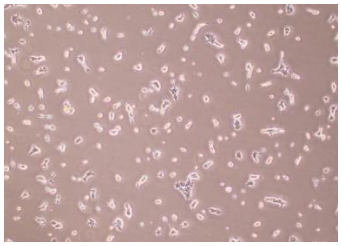
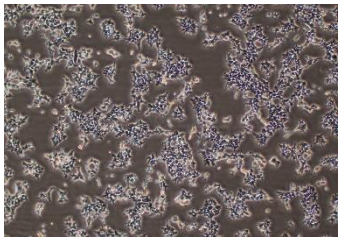
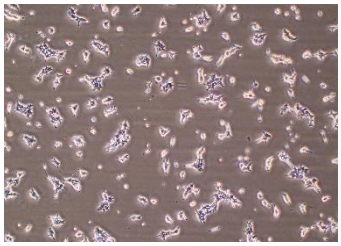
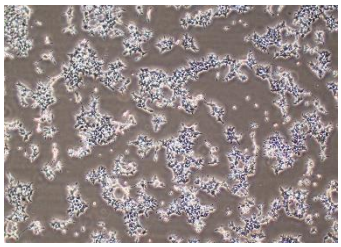
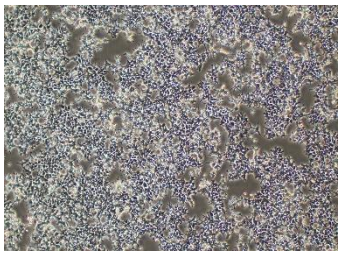
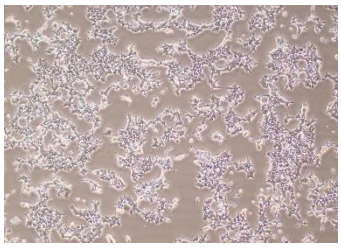
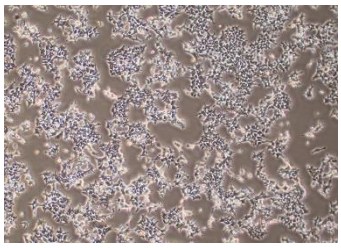
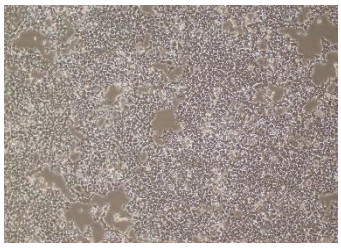
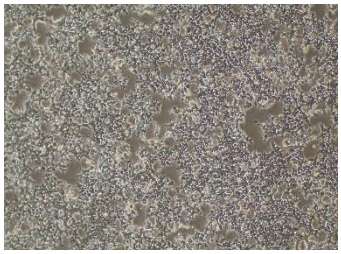


Figure 2. Cell viability of HEK293T cultured in CCL-170B-8-TS incubator over 5 days of incubation at different passages (P2, P4, P5).

Table 1. Representative microscopic images showing the growth and morphology of HEK293T cultured in CCL-170B-8-TS at various incubation times and passages.

Days	Passage 2 (P2)	Passage 4 (P4)	Passage 5 (P5)
Day 2			
Day 3			
Day 4			
Day 5			

Microscopic observations (Table 1) further confirmed the quantitative growth trends. On day 2, cells across all passages remained at relatively low densities with rounded morphology, reflecting early adaptation. From day 3 to day 4, cell numbers increased markedly, and morphology transitioned into elongated and adherent forms, with P4 showing slightly faster proliferation. By day 5, near-confluency was achieved in all passages, with healthy and tightly packed cells. These findings support the cell count and viability data, indicating that HEK293T cells cultured in CCL-170B-8-TS maintained robust morphology and proliferative capacity during serial passaging.

Conclusion

The growth and viability assessment of HEK293T cells demonstrated that CCL-170B-8-TS incubator provides a stable and supportive environment for maintaining cell health. Cell proliferation progressed consistently, reaching near-confluency by day 5, while viability remained high (92–97%) across passages, indicating minimal stress during culture. Overall performance suggests that the incubator effectively supports long-term culture with reliable outcomes for cell-based applications.

References

1. R. Lehmann, J.C. Severitt, T. Roddelkopf, S. Junginger, K. Thurow, Biomek cell workstation: a variable system for automated cell cultivation, *J. Lab. Autom.* 21 (3) (2016) 439e450, https://doi.org/10.1177/2211068215599786.
2. Mulyazmi, Mulyazmi & Wan Daud, Wan & Octavia, Silvi & Ulfah, Maria. The Relative Humidity Effect Of The Reactants Flows Into The Cell To Increase PEM Fuel Cell Performance. *MATEC Web of Conferences.* 2018. 156. 03033. 10.1051/matecconf/201815603033.
3. Triaud, F.; Clenet, D.; Cariou, Y.; et al. Evaluation of Automated Cell Culture Incubators. *J. Assoc. Lab. Autom*. 2003, 8, 82–86.
4. Klein SG, Steckbauer A, Alsolami SM, Arossa S, Parry AJ, Li M and Duarte CM (2022) Toward Best Practices for Controlling Mammalian Cell Culture Environments. *Front. Cell Dev. Biol.* 10:788808. doi: 10.3389/fcell.2022.788808