Performance Evaluation of Esco CelCulture® Incubator (CCL-TS)

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Summary

The CCL-170T-8-TS incubator fully complies with YY1621-2018 and DIN 12880 standards, demonstrating excellent temperature stability (fluctuation 0.05–0.08 °C, uniformity 0.31 °C) and rapid recovery after door openings (temperature 5 min; CO_2 ≤5 min and O_2 ≤10 min). With exceptionally low gas fluctuations (CO_2 ±0.03%, O_2 ±0.02%), the system ensures a highly stable and reproducible microenvironment that supports robust cell growth and viability

***Key Words**: CelCulture® CO_2 Incubators Touch Screen, fluctuation, uniformity, recovery*

Introduction

Cell culture incubators play a critical role in providing a controlled environment that supports optimal cell growth and viability. The accuracy and stability of parameters such as temperature, carbon dioxide (CO_2), and oxygen (O_2) are essential for ensuring reproducible and reliable experimental outcomes. Standard condition for cell culture is temperature of 37°C, atmospheric air (21% volume fraction of O_2) enriched by 5% CO_2, and humidity provided by spontaneous water evaporation[1].

Accurate temperature control and uniform heat distribution are essential to prevent thermal gradients that may compromise cell proliferation. In addition, maintaining 5% CO_2 is critical for stabilizing the pH of culture media, as most formulations rely on a sodium bicarbonate buffering system that interacts with CO_2 gas[2]. Equally important is the regulation of oxygen, since cell cultures require physiological oxygen levels (physioxia). Without proper O_2 control, cultures are exposed to hyperoxia conditions that can trigger excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), ultimately affecting cell health and experimental outcomes[3].

Minor fluctuations and uniformity in cell culture incubators are commonly caused by door openings and slow recovery of heating and gas control systems[4]. These disruptions lead to oxidative stress, such damaging lipids, protein, and DNA[5]. For this reason, evaluating the performance of cell culture incubators in terms of stability, uniformity, and recovery is essential to ensure consistent culture conditions and to support reproducible scientific outcomes.

Materials and Method

Materials used in this experiment were:

1. Esco CelCulture® Touch CO_2 Incubator (CCL-170T-8-TS SN-200851)
2. Esco CelCulture® Touch CO_2 Incubator (CCL-170T-8-TS SN-200852)
3. Esco CelCulture® Touch CO_2 Incubator (CCL-170T-8-TS SN-200848)
4. G100 CO2 Analyzer
5. Keysight DAQ970A
6. Keysight DAQ970A
7. Yokogawa DX2048
8. AC Power Supply
9. AC Power Supply

Temperature Fluctuation

Temperature fluctuation measurements were conducted using 27-channel type T thermocouples, positioned at the upper, middle, and lower trays (nine point for each tray) to represent the entire chamber volume. After a stabilization period of at least 3 hours after door opening, temperature data were continuously recorded for 1 hour at an interval of ≤ 1 minute. For each sensor, the maximum (T_{max}) and minimum (T_{min}) temperatures during the measurement period were determined. Temperature fluctuation was then calculated using the formula:

$$\Delta Tfluct, channel = \pm \frac{Tmax - Tmin}{2}$$

Temperature Uniformity

Temperature uniformity was evaluated by first calculating the average temperature of each channel over the 1-hour acquisition period ($T_{avg,channel}$). These average values were then corrected with an offset, if necessary, to account for sensor calibration differences, resulting in more accurate average corrected data. From the set of 27 average corrected values, the highest ($T_{avg,max}$) and lowest ($T_{avg,min}$) averages were identified as the basis for uniformity assessment within the chamber. Uniformity was then calculated using the formula:

$$\Delta Tuniformity = \pm \frac{Tavg, max - Tavg, min}{2}$$

Temperature Recovery

Temperature recovery was assessed by first stabilizing the incubator for 3 hours at 37 °C. Once stabilized, the door was opened at a 90° angle for 30 seconds and then closed. After the door was closed, the recovery time was recorded, defined as the duration required for the chamber return to 98% from initial setpoint after the door closed. Data was collected using the unit's internal datalogger.

CO_2 Fluctuation

The measurement of CO_2 fluctuation was carried out after stabilizing the incubator for a minimum of 3 hours. Following stabilization, CO_2 concentrations were continuously recorded over a 1-hour period with a sampling interval of ≤ 1 minute. The maximum (CO_{2max}) and minimum (CO_{2min}) values within the acquisition period were identified. The fluctuation was subsequently calculated using the following formula:

$$\Delta Tfluct, CO_2 = \pm \frac{CO_2\, max - CO_2 min}{2}$$

CO_2 Recovery

The measurement of CO_2 recovery was conducted after stabilizing the incubator for a minimum of 3 hours at 5% CO_2. Following stabilization, the chamber door was opened to a 90° angle for 30 seconds and subsequently closed. The recovery time was then determined, defined as the duration required for the CO_2 concentration to return to the set point of 5%. All data were recorded using the unit's internal datalogger

O_2 Fluctuation

The measurement of O_2 fluctuation was carried out after stabilizing the incubator for a minimum of 3 hours. Following stabilization, CO_2 concentrations were continuously recorded over a 1-hour period with a sampling interval of ≤ 1 minute. The maximum (O_{2max}) and minimum (O_{2min}) values within the acquisition period were identified. The fluctuation was subsequently calculated using the following formula:

$$\Delta T fluct, O_2 = \pm \frac{O_2\, max - O_2 min}{2}$$

O_2 Recovery

The measurement of O_2 recovery was conducted after stabilizing the incubator for a minimum of 3 hours at 5% O_2. Following stabilization, the chamber door was opened to a 90° angle for 10 minutes and subsequently closed. The recovery time was then determined, defined as the duration required for the O_2 concentration to return to the set point of 5%. All data were recorded using the unit's internal datalogger

Results and Discussion

The performance of a cell culture incubator is a critical determinant of experimental reproducibility and the reliability of biological outcomes. The incubator's performance was evaluated with reference to two key standards: YY1621-2018 (Medical Carbon Dioxide Incubator), which defines criteria for temperature stability, recovery, and CO_2 control in supporting cell culture, and DIN 12880 (Laboratory Ovens and Incubators), which specifies universal metrics such as temperature fluctuation, uniformity, and recovery. Aligning the results with both standards allows evaluation of the incubator's reliability from both biological and technical perspectives.

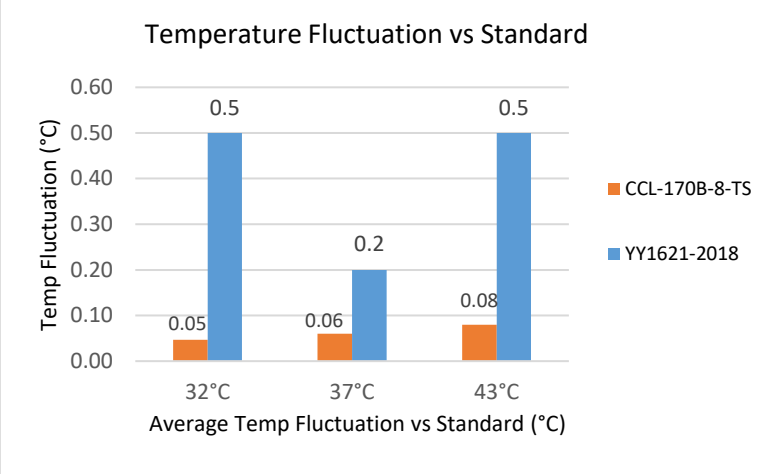

Figure 1. Temperature Fluctuations

Temperature fluctuation on CCL-170T-8-TS was evaluated at three representative setpoints: 32 °C, 37 °C, and 43 °C. The 32 °C setpoint was selected as a representative condition for the lower operating range (23–36.9 °C), while the 43 °C setpoint was used to represent the higher operating range (37.1–60 °C). The 37 °C setpoint was chosen as the critical condition for mammalian cell culture applications. The acceptance criteria were defined according to YY1621-2018, with maximum allowable fluctuations of ≤0.5 °C in range 23–36.9 °C and 37.1–60 °C, and ≤0.2 °C at 37 °C.

Across all test conditions, the CCL-170T-8-TS demonstrated fluctuations well below the specified limits. At 32 °C, the measured fluctuation was 0.05 °C, representing only 10% of the maximum allowable value. At 37 °C, fluctuation was 0.06 °C, significantly lower than the 0.2 °C threshold. At 43 °C, fluctuation was 0.08 °C, again far below the 0.5 °C limit. These fluctuation values, being

substantially below the specified thresholds, align with previous research indicating that minor temperature variations within this range (≤0.11°C) exert no significant impact on mammalian cell morphology, viability, or proliferation dynamics, thereby validating the incubator's suitability for supporting reliable cell culture outcomes[3].

Table 1. Temperature Uniformity and Recovery on CCL-170T-8-TS

Parameter	Conditions	Average	Product Requirement
Temp Uniformity (°C)	Temp chamber 37°C	0.31°C	± 0.4°C
Temp Recovery (minutes)	Door open 90° for 30s	5 minutes	± 5 minutes
	Temp chamber to reach 98% from initial setpoint (36,6°C)		

The temperature performance of the CCL-170T-8-TS was evaluated under controlled operating conditions. At a chamber set point of 37 °C, the incubator achieved a uniformity of 0.31 °C, which is well within the acceptance criteria of ±0.4 °C. This stable distribution ensures minimal variability across the chamber, thereby reducing potential experimental bias. Supporting evidence from previous studies indicates that even a thermal deviation of ±0.3 °C is sufficient to maintain high cell viability, with chondrocytes reaching 95±2% viability after three days of culture[6]. Accordingly, the temperature uniformity observed in the CCL-170T-8-TS can be considered biologically appropriate for sustaining healthy cell growth.

In addition, recovery performance was evaluated by opening the chamber door at 90° for 30 seconds, followed by measurement of the time required for the chamber return to 98% from initial setpoint after the door closed. The recovery time was 5 minutes, aligning exactly with the acceptance limit of ±5 minutes. A temperature recovery time of five minutes has been validated as a critical factor in supporting early-stage embryo development and blastocyst formation in cell culture experiments[7]. This indicates that the instrument is capable of rapidly re-establishing stable conditions after disturbance, an essential factor for ensuring reproducibility in sensitive applications.

Table 2. CO_2 Fluctuation and Recovery on CCL-170T-8-TS

Parameter	Conditions	Average	Product Requirement
CO_2 Fluctuation (%)	Temp chamber 37°C	0.03%	± 0.2%
	Set point CO_2 level 5%		
CO_2 Recovery (minutes)	Door open 90° for 30s	4 minutes	≤ 5 minutes
	The chamber condition reached 98% of the CO_2 setpoint from the initial value (5,2%)		

Stability is critical for maintaining the pH of culture media, as even small deviations in CO_2 can alter bicarbonate buffering and subsequently affect cell physiology[2].The CCL-170T-8-TS incubator demonstrated excellent CO_2 gas stability and recovery performance. The CO_2 fluctuation was maintained at ±0.03%, which represents only 15% of the maximum allowable deviation (±0.2%) and highlights the precision of the gas regulation system. Previous studies have reported that CO_2 oscillations of ±0.5% can already induce medium pH shifts and morphological changes[8], therefore the minimal fluctuations observed here are unlikely to exert measurable biological effects, ensuring a highly stable culture environment that supports reproducible growth and phenotypic stability.

Furthermore, the CO_2 recovery time of 4 minutes after door opening complied with the product requirement of ≤ 5 minutes, ensuring rapid restoration of the optimal culture environment. While recovery times of 11–15 minutes have been associated with medium pH perturbations and potential stress to cultured cells, the 4-minute recovery observed in this incubator is unlikely to exert any significant adverse effects on cell culture[4].

Table 3. O_2 Fluctuation and Recovery on CCL-170T-8-TS

Parameter	Conditions	Average	Product Requirement
O_2 Fluctuation (%)	Temp chamber 37°C	0.02%	± 0.2%
	Set point O_2 level 5%		
O_2 Recovery (minutes)	Door open 90° for 30s	10 minutes	≤ 10 minutes
	The chamber condition reached 98% of the O_2 setpoint from the initial value (4,9%)		

Based on the Table 3, the CCL-170T-8-TS incubator exhibited highly stable oxygen control, with an O_2 fluctuation of only ±0.02%, far below the allowable limit of ±0.2%. Moreover, previous studies have shown that even O_2 fluctuations ±0.02% cell viability consistently remained 90% cell viability[3]. This stability reflects precise control of gas delivery, which is critical for maintaining hypoxic or physiologically relevant oxygen environments in cell culture.

Regarding dynamic performance, the O_2 recovery time was measured at 10 minutes after a 90° door opening for 30 seconds, aligning with the maximum limit specified by the product requirement (≤10 minutes). Recovery times of 11–15 minutes have previously been associated with medium pH perturbations and potential stress to cultured cells[4]. Therefore, maintaining O_2 recovery times below this threshold is critical to preserving a stable culture environment, as transient re-oxygenation events have been shown to modulate reactive oxygen species (ROS) production and alter gene expression profiles[5].

Conclusion

The performance evaluation of the CCL-170T-8-TS incubator demonstrated compliance with both YY1621-2018 and DIN 12880 standards, confirming its reliability in controlled cell culture applications. Temperature stability was well maintained, with fluctuations (0.05–0.08 °C) and uniformity (0.31 °C) remaining within acceptance limits, ensuring minimal variability across the chamber. The recovery times following door openings complied with the specified performance criteria, with CO_2 concentration re-established within ≤5 minutes and O_2 concentration within ≤10 minutes. Furthermore, gas regulation accuracy was validated by exceptionally low CO_2 (±0.03%) and O_2 (±0.02%) fluctuations, far below the maximum allowable thresholds.

Collectively, these results confirm that the CCL-170T-8-TS provides a stable and reproducible microenvironment, thereby supporting robust cell viability and growth. Such performance highlights the incubator's suitability for a wide range of cell culture applications, ensuring consistency with commercial-grade systems while fully meeting international standards.

References

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