Cell Locker System segregates stem cells, protecting from contamination and enhancing environmental stability

Mary Kay Bates, Jürgen Schneider, Molly Love, and Lindsey Low,
Thermo Fisher Scientific

Abstract

Delicate cultures including stem cells and other primary cells are increasingly used in cell therapy research and development for applications in oncology, immunology, neurology and more. Commonly, these cells require daily manipulation, potentially exposing them to contamination. Such cells are also uniquely reactive to environmental variation. To address these challenges, we developed the Thermo Scientific™ Cell Locker™ System, a new CO_2 incubator design which includes six individual transparent Cell Locker Chambers. Tests show the Cell Locker System provides enhanced stability, contamination control and flexibility for isolation of labile cell types or sensitive projects in a shared incubator. Each Cell Locker Chamber features dual 0.2 µm membrane filters which allow air and humidity exchange but prevent transmission of microorganisms. Functional studies show that when one Cell Locker Chamber is opened, remaining chambers in the Cell Locker System provide environmental stability for temperature, humidity, and CO_2/O_2 atmosphere, maintaining ideal conditions not possible in a standard incubator. Gas use and media evaporation are both reduced by 50%. Multiple clones of induced pluripotent stem cells from different donors were evaluated and found to be indistinguishable from control incubators where conditions and manipulations had previously been optimized, showing that the Cell Locker System design and materials facilitated optimal cell growth. The Cell Locker System represents a novel approach for culturing stem cells and other sensitive cultures used in cell therapy applications.

Introduction

Stem cells tend to be less resilient, more fragile and more sensitive than immortalized cell lines which have been growing in culture for many decades. Delicate stem cells are less resilient to contamination, compared to immortalized cells. Stem cells typically require daily manipulation and care; significantly more manipulation than traditional cell lines. This daily handling puts the cells at risk of exposure[1] to contamination from the air and from lab worker's normal flora[2], more so than cell lines which may only require handling every third day. Also, the growth media and reagents used for culturing stem cells are significantly more expensive – generally at least ten times more – than for standard cell types such that any contamination is more costly when stem cells have to be discarded and experiments repeated. And stem cells are more sensitive to changing conditions; by their nature they are reactive to triggers which could signal them to differentiate and lose pluripotency. Primary cells including

ThermoFisher SCIENTIFIC

stem cells may carry contaminants from the source tissue or primary isolation process[2] that could spread to other cultures. Cultures brought into the lab should be quarantined until they can be tested to ensure they do not harbor microbial contaminants including mycoplasmas. Because of their sensitivity, most labs recommend that stem cells be segregated long-term in a different incubator than other cell types. Unfortunately, dedicating an entire incubator to quarantine or segregate a few cultures is not always possible due to space limitation in the lab, or to limited funding to pay for another cell culture incubator.

To improve culturing of stem cells and other delicate cultures, Thermo Scientific™ Cell Locker™ System is a novel innovation designed into the Thermo Scientific™ Heracell™ VIOS™ and Thermo Scientific™ Forma™ Steri-Cycle™ 160 liter CO_2 Incubators. As shown in Figure 1, the Cell Locker System subdivides the traditional incubator into six polycarbonate chambers which can stay in the incubator or can slide out. Each Cell Locker Chamber has its own small door but the full incubator inner door can also be opened for full access cleaning. The incubator itself features a proven 12-log dry heat sterilization cycle[3], in-chamber HEPA filtration and sensors, and covered, protected water reservoir. The entire incubator chamber can also be composed of 100% pure copper. Each Cell Locker Chamber is made of the same type of polycarbonate that makes up most culture dishes, and includes an optional transport door in case users want to carry the chamber out of the incubator, to protect cells from the incubator to the hood, or to another lab down the hall. Cell Locker Chambers are autoclavable at 121˚C up to twelve times. Both sides of each Cell Locker Chamber feature an easily replaceable 0.2 micrometer (µm) membrane filter which permits air circulation, but prevents transmission of particles into or out of the Cell Locker Chambers when the door is closed, making each Cell Locker Chamber a quarantine incubator for segregating projects, users, or cell types.

Figure 1: Cell Locker System Segregates Cultures or Projects. Cell Locker System consists of six individual protected chambers inside a Heracell VIOS 160i or a Forma i160 Steri-Cycle CO_2 incubator. 0.2 µm filter membranes permit air exchange but protect from cross-contamination and maintain ideal conditions.

The Cell Locker System is easily configured to fit the way your lab works. Use it to segregate different cell types, users, or experiments, to isolate and protect them from other projects. For example, if you previously had one incubator that was only for induced pluripotent stem (iPS) cells, now you can keep these cells in the same incubator with your other cell types while segregating them, reducing the number of incubators needed and saving space in the laboratory.

Each Cell Locker Chamber holds nine T-75 flasks or up to twenty-eight micro-well plates. An optional sliding work tray offers easy access to flasks, dishes or plates. See Figure 2.

Figure 2: Each Cell Locker Chamber features dual 0.2 µm membrane filters which permit air exchange but restrict microorganisms from entering or exiting. The chamber will hold nine T-75 flasks or up to twenty-eight micro-well plates.

Cell Locker System segregates cultures and prevents entry of contaminants

Cell culture contamination is an ongoing challenge for anyone working with cells. Nearly everyone will experience at least one contamination event each year, and many researchers have more than one. Many of these contaminants come from us. Humans carry an average of 10,000 microorganisms/cm^2 on our skin.[4] Combining our normal flora with microorganisms in the environment, even indoor air carries 30-1000 microorganisms/m^3.[5] Mycoplasma species are the most common cell culture contaminant, with up to 15% of cultures in the United States harboring these bacteria.[6] In other regions, mycoplasma contamination rates are even higher, with up to 80% contamination in Japan, and an average of 35% in Europe and the rest of the world.[7]

Mycoplasmas cause widespread effects. Because of their ubiquity and their effects on cells, they are the most serious contaminant for cultured cells. These tiny bacteria affect the host cell metabolism, retard growth, and may affect morphology.[8] They can cause chromosomal alterations and damage DNA, and provoke cytopathic responses.[9] All of these alterations mean that cells will not respond normally in downstream analyses. Broad spectrum antibiotics commonly used in culture media are ineffective against mycoplasmas[6], so the only way to battle them is to test regularly. Statistics show that the incidence of mycoplasma contamination is inversely proportional to the amount of testing, because early detection can limit the spread and the damage.[7]

Trying to determine the cause of poor cell viability while a mycoplasma outbreak spreads across a lab can be very time consuming and costly. One lab said they spent as much as $100,000 U.S. fighting a mycoplasma outbreak.[10] In a more typical example, consider a stem cell researcher who might spend one month dealing with a mycoplasma contamination of her human stem cells. A new vial of cells costs $1000, and a mycoplasma test kit costs $300. She may spend more than $2000 on materials and reagents over that month to test and repeat experiments. Her salary for one month may be $4000, and meanwhile all her other work stopped while she worked to eliminate this contamination. Such examples point to the value of the Cell Locker System, to protect cells from contamination.

Independent tests performed by two commercial test labs showed that no microorganisms were able to enter or exit a closed Cell Locker Chamber.[11] Fifteen rounds of testing were performed, using three very small bacteria. *Staphylococcus aureus* is about 0.8 µm in diameter, *Brevundimonas diminuta* is about 0.2 µm, and is the accepted test organism for 0.2 µm pore size, and *Mycoplasma orale* is one of the most common cell culture contaminants, and is 0.1-0.6 µm in size.

In all these tests, summarized in Table 1, not one bacterial colony -- no contamination -- was able to enter a closed Cell Locker Chamber. Yet hundreds of microorganisms were collected outside the Cell Locker Chambers, inside the incubator. More information on these and other tests is provided elsewhere.[11]

Table 1. In multiple tests using very small bacteria, all growth media remained sterile inside the Cell Locker Chambers, while hundreds of microbes were collected outside the Cell Locker Chambers but inside the incubator, showing that microbes cannot penetrate a closed Cell Locker Chamber.

Cell Locker System Design Prevents Entry of Microorganisms to Cell Locker Chambers

Cell Locker Chamber	Circulated Microorganism Concentration					
	Staphylococcus aureus **ATCC 6538** **9.6 x 10^4 CFU**		***Brevundimonas diminuta*** **ATCC 19144** **1.6 x 10^5 CFU**		***Mycoplasma orale*** **DSM 25590** **9.3 x 10^4 CFU**	
Location	Inside	Outside Top	Inside	Outside Top	Inside	Outside Top
Top left	0	>300	0	78	0	>300
Top right	0	>300	0	75	0	>300
Middle left	0	>300	0	112	0	>300
Middle right	0	>300	0	111	0	>300
Bottom left	0	>300	0	36	0	>300
Bottom right	0	>300	0	37	0	>300

Cell Locker System provides unparalleled environmental stability

Cells of all types are constantly sampling their external environment, and continuously responding to conditions and changes in temperature, atmosphere, humidity, nutrients, and stimulants or repressors in the medium. Cellular responses will change depending on these cues. So variations in any of these can cause varied responses in differential gene or protein expression, or whether the cells enter the growth cycle, grow faster or slower, or stay quiescent. The second goal of the Cell Locker System is to maintain the environmental conditions as much as possible, even during a door opening.

Consider what happens in the Cell Locker System compared to a normal incubator, when opening the door to remove cultures. Closed Cell Locker Chambers remain stable when a neighboring Cell Locker Chamber is opened. For example, if primary hepatocytes are incubated in one Cell Locker Chamber and iPS cells in another, the iPS cells would experience no drop in temperature, CO_2 or humidity when the primary cells are removed for passaging or analysis. Figure 3 summarizes what happens when a typical stem cell incubator door is opened in a typical laboratory, compared to what happens in a closed Cell Locker Chamber when a neighboring chamber is opened. In the laboratory, the room temperature is 20-22˚C, atmospheric CO_2 concentration is 0.04%, 30-60% relative humidity, and atmospheric oxygen of 21%.

In the Heracell VIOS Incubator, the environment for stem cells would typically be 37˚C, 5% CO_2, 93% humidity or higher, and let's say 1% oxygen. But when you open the incubator door, the conditions inside flow out to mix with the lab air, and after a thirty second door opening, the incubator temperature drops to about 30˚C, CO_2 drops to below 1%, relative humidity to below 30%, and oxygen concentration approaches atmospheric 19-21%. All of these conditions except hypoxia will be re-established to the Heracell VIOS incubator pre-set conditions in ten minutes following this thirty second door opening, although in most incubators the recovery is much slower.[12] Restoring the 1% O_2 will take more than 20 minutes. For sensitive cells such as stem cells, this large change in conditions accompanying the door opening can be disruptive. This effect is multiplied every time the incubator door is opened. Thus, if we extend the example to a shared incubator with multiple users, it is clear that during a typical day cultured cells may not spend much time at their ideal conditions.

Now let's look at what happens in the Cell Locker System. When one Cell Locker Chamber is opened, the closed chambers maintain 37 degrees, 5% CO_2, 93% humidity, and oxygen remains very low at 1-3%, as shown in Figure 3.

Figure 3: A typical laboratory has conditions far different than in a typical CO_2 Incubator. During a door opening, conditions in any incubator drop close to those in the surrounding room. In this example, the CO_2 incubator is set to 37˚C, 5% CO_2, 1% oxygen, 93% humidity but these parameters drop swiftly during a thirty second door opening. In a Cell Locker System, ideal conditions are maintained when a neighboring Cell Locker Chamber is opened to remove cultures.

Typical Laboratory
Conditions:
22°C
0.04% CO_2
30-60% humidity
21% oxygen

Standard Heracell VIOS 160i CO_2 Incubator
Conditions during door opening:
Temperature below 30°C
CO_2 below 1%
Humidity below 30%
Oxygen briefly 19-21%

Closed chambers in Cell Locker System when one chamber is opened
Maintains:
37°C ± 0.3°C
5% CO_2 ± 0.2%
≥93% Humidity ± 3%
1-3% oxygen

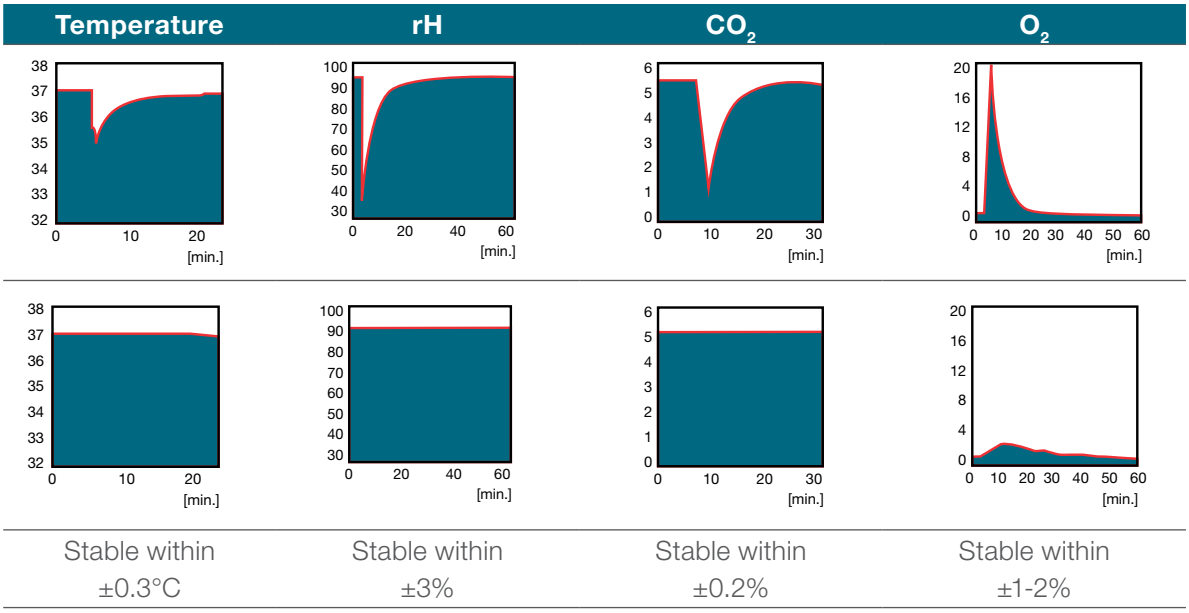
The stability of the five unopened Cell Locker Chambers when one chamber is opened is more clearly demonstrated when shown in graphic form of the conditions over time. The top row in Figure 4 shows the recovery in minutes when opening one Cell Locker Chamber for 30 seconds, a very long door opening. In the opened chamber, the temperature drops and rapidly recovers to 37˚C. A similar fast recovery happens for relative humidity and CO_2. Oxygen concentration in the opened Cell Locker Chamber rises rapidly to approach atmospheric 21% and then rapidly drops back down to 1%.

The bottom row in Figure 4 shows the conditions in the Cell Locker Chambers which were not opened. The time course shows a stable, flat line for temperature, relative humidity, and CO_2. The oxygen shows a slight bump of 1-2% above 1%, but this is much better for stem cells than rising all the way to 21%. This is an extreme example of culturing at 1% oxygen, but most stem cell scientists are probably culturing at 3-5% oxygen and would not see much of a change at all. For sensitive stem cells, this provides a much more stable environment than culturing in a standard incubator. More information and data on the stability and recovery of the Cell Locker System can be found in an online e-book.[13]

Figure 4: When one Cell Locker Chamber is opened, the chamber temperature, CO_2, humidity and O_2 approach the conditions of the surrounding room, as they would when any incubator door is opened. In contrast, the closed Cell Locker Chambers remain at or near stable set conditions.

Opened Cell Locker Chamber

Unopened Cell Locker Chamber



Cell Locker System saves gas and reduces evaporation

Another benefit of the Cell Locker System is that it reduces gas use and evaporation by at least 50%, depending on the set conditions, compared to the same incubator design not incorporating the Cell Locker System.

When using nitrogen gas to create hypoxic conditions in an incubator, maintaining oxygen concentration at 1-6% instead of atmospheric 21% uses a lot of nitrogen gas (N_2). As shown in Table 2, if the incubator doors are never opened, the Cell Locker System uses slightly more gas than the standard incubator because all the gaskets in the small doors let a little gas leak through. But in a more realistic scenario where lab workers are opening the doors throughout the day, the Cell Locker System uses at least 50% less gas, depending on the number of openings and the desired oxygen concentration. In the most common example where the door is opened six times for 30 seconds, using 3% oxygen, the Cell Locker System uses 60% less nitrogen. So for a typical lab, this 8,500 liter tank of nitrogen gas would last 14 days instead of 5.7 days, saving significant cost. CO_2 gas use was also reduced by about 50%, but much less CO_2 gas is used compared to N_2 since in general a CO_2 gas tank will last up to two months in a typical lab (results not shown).

Table 2. Comparison of nitrogen gas usage per day in a standard hypoxic incubator compared to a hypoxic Cell Locker System shows that in a typical stem cell laboratory day of six 30-second door openings at 5% CO_2 and 3% O_2, the Cell Locker System uses 60% less N_2.

Activities per day	N_2 gas consumption per day	
	Standard CO_2/O_2 Incubator	Cell Locker System
No door opening at 3% O_2	70 L	120 L
One x 30 sec. door opening at 3% O_2	236 L	80 L
Three x 30 sec. door openings at 3% O_2	778 L	360 L
Three x 30 sec. door openings at 5% O_2	548 L	224 L
Three x 30 sec. door openings at 1% O_2	1325 L	840 L
Six x 30 sec. door openings at 3% O_2	1486 L	600 L

Due to the smaller enclosures and engineered airflow, the Cell Locker System reduces evaporation of growth media by over 50%, compared to a standard CO_2 incubator, as shown in Figure 5. Tests were performed using 15 grams (g) of water in each of three 10-centimeter Petri dishes in all six Cell Locker Chambers. The average evaporation rate was of 0.6 g of water loss in 24 hour period with no door openings. That compares to a standard Heracell VIOS incubator, which itself has very low evaporation, with a rate of 1.3 g of water loss per day. In each Cell Locker Chamber, there was even less evaporation at the back of the chamber than next to the door. For researchers using 96-well and 384-well screening assays where edge effect or drying is affecting readout results, the Cell Locker System offers a 50% reduction in evaporation, providing greater clarity and tighter replicates for faster experimental conclusions.

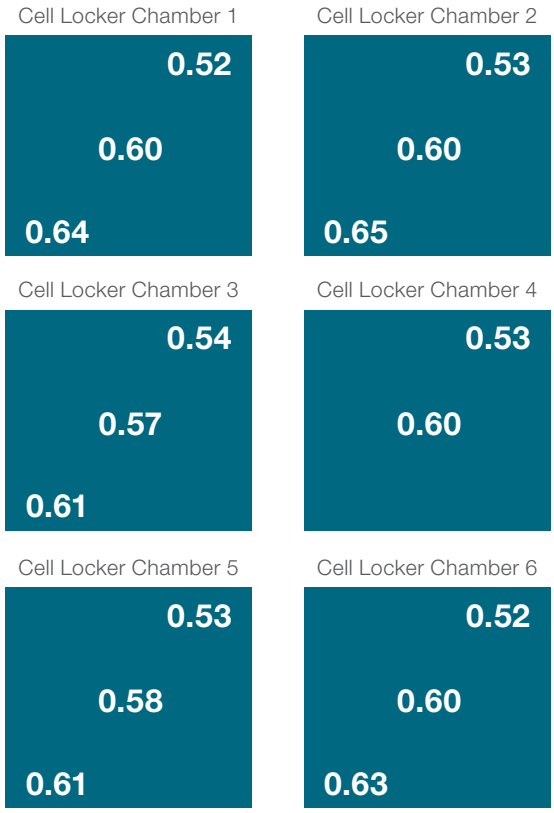


Figure 5: In a Cell Locker Chamber, loss of water due to evaporation is 0.6 g/day from open dishes with 15 g total water. Each square represents one Cell Locker Chamber in the Cell Locker System incubator and the three numbers in each square represent the evaporation from each of three Petri dishes in a 24 hour period. In comparison, the same test performed in a standard CO_2 incubator without the Cell Locker System showed an average loss of 1.3 g/day (results not shown).

iPS cells show ideal growth characteristics

In a lab producing induced pluripotent stem cells (iPS cells) and later differentiating them into desired cell types, a new Cell Locker System was tested side by side with a previously optimized incubator. The cells were derived from four different donors, split into two populations and grown in parallel in the two different incubators.[14] The iPS cells showed the same quality when cultured in the Cell Locker System compared to an optimized incubator. In both cases more than 50 colonies were tested and compared, and zero spots of differentiation were found. Table 3 summarizes the results. All parallel cultures grew at the same rate, and all passages were performed on the same day.

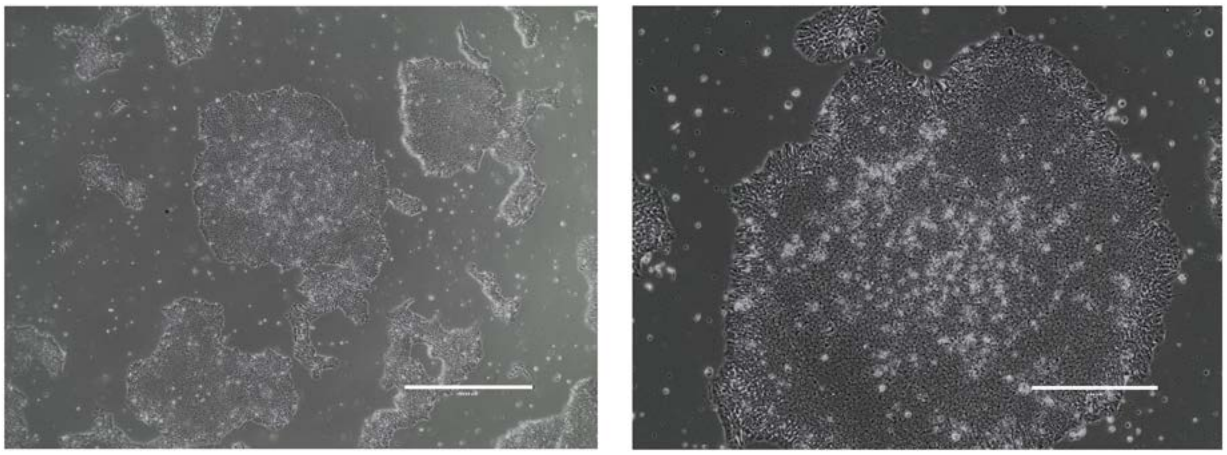
Table 3. iPS cells had the same quality following culture in a Cell Locker System compared to an optimized incubator. Cells were derived from four different donors, split into two populations and grown in parallel in the two incubators. Procedures were developed using the Thermo Scientific Forma 3130 water jacketed incubator.

iPS Cells	Cell Locker System	Thermo Scientific Forma Incubator
Colonies observed	>50	>50
Spots of differentiation	None	None

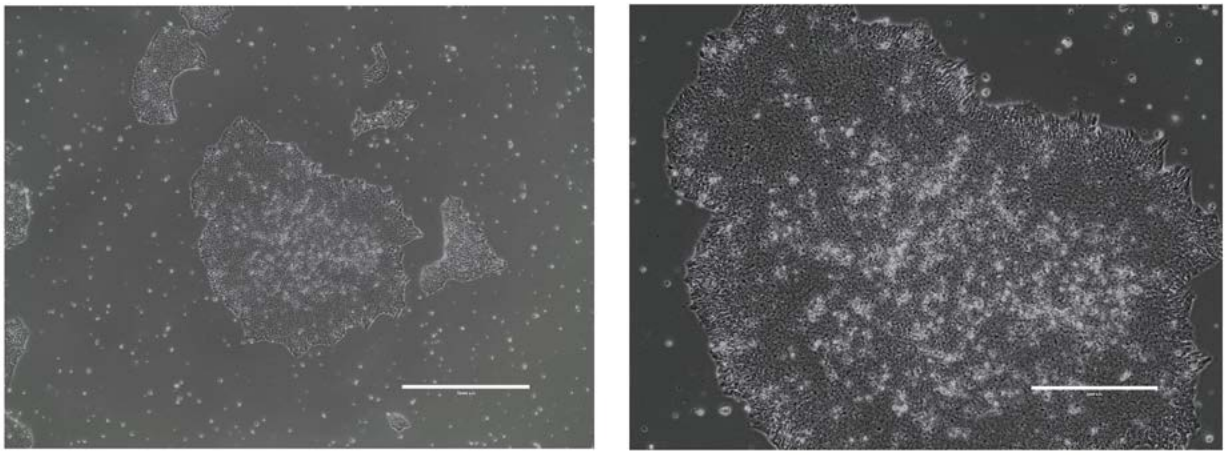
Next, cells from each system were harvested and cryopreserved. When cells in the optimized system and in the Cell Locker System were thawed, re-plated and assessed, the iPS cell colonies showed the same quality regardless of the incubation, as shown in Figure 6. This work shows that the materials and environmental conditions in the Cell Locker System can, out of the box, provide the ideal conditions obtained after a lot of time and investment in a carefully designed system.

Figure 6: Typical iPS cell colonies show the same quality following cryopreservation and regrowth in the Cell Locker System (A) and in an optimized incubator (B).

A. iPS cell colonies from Cell Locker System



B. iPS cell colonies from optimized incubator



thermo scientific

Conclusions

The Cell Locker System represents a novel approach to providing improved culturing conditions for sensitive cell types such as primary cells, stem cells, and more. This patented design maintains desired conditions so that cells spend more time in an environment that mimics physiological conditions. Under such conditions, cultured cells will provide responses that better model those in the intact organism, for better predictions of human responses for drug and disease modeling for studies in regenerative medicine, cell therapy, oncology, neuroscience, immunology and more. Microorganisms are prevented from entering closed Cell Locker Chambers. Compared to a CO_2 incubator which itself has outstanding conditions, the Cell Locker System remains more stable, recovers faster, shows 50% less evaporation and uses 50% less gas even under hypoxic conditions. Stem cells showed the same ideal characteristics in the Cell Locker System as in an optimized traditional incubator.

References

1. Inamdar MS, Healy L, Sinha A. Global solutions to the challenges of setting up and managing a stem cell laboratory. Stem Cell Rev and Rep 8, 2012.
2. Cobo F, Cortés JL, Cabrera C, Nieto A, Concha A. Microbiological contamination in stem cell cultures. Cell Biol Int 31, 2007.
3. Thermo Fisher Scientific. Thermo Scientific Steri-Run sterilization cycle proves total sterilization. ANCO2STERIRUN 0215, 2015.
4. Grice, EA et al. A diversity profile of the human skin microbiota. Genome Res 18, 2008.
5. Stryjakowska-Sekulska M et al. Microbiological quality of indoor air in university rooms. Polish J Environ Stud 16(4), 2007.
6. Drexler HG, Uphoff, CC Mycoplasma contamination of cell cultures: Incidence, sources, effects, detection, elimination, prevention. Cytotechnology. 39 (75), 2002.
7. Ryan J. Understanding and managing cell culture contamination. Corning Inc. Life Sciences, 2008.
8. Nikfarjam L and Farzaneh P. Prevention and detection of mycoplasma contamination in cell culture. Cell J 13(4), 2012.
9. Rottem S, Kosower NS and Kornspan JD. Contamination of tissue cultures by mycoplasmas. In Biomedical Tissue Culture, chapter 3, InTech 2012.
10. Callaway E. Contamination hits cell work. Nature 511, 2014.
11. Bates MK, Love Parrucci M. Thermo Scientific Cell Locker System prevents entry of microorganisms for protection of sensitive cell cultures. Thermo Fisher Scientific ANCELLLOCKER 0119, 2019.
12. Thermo Fisher Scientific. Smart Note: Which incubation parameters are most important for proper cell growth and expression? PFCO2SMARTNOTE 0715, 2015.
13. Bates MK, Schneider J, Love Parrucci M. Protected chambers for your most sensitive cells. https://s3-us-west-1.amazonaws.com/tfs.dig/COL17064-Cell_Locker_Solutions-r/index.html Thermo Fisher Scientific, 2019.
14. Bates MK, Schneider J, Love Parrucci M. Thermo Scientific Cell Locker System segregates stem cells and other delicate cultures, protecting from contamination and enhancing environmental stability in a shared incubator. Presented at Bioprocessing International conference, Boston MA, 2018.

Find out more at **thermofisher.com/celllocker**

ThermoFisher SCIENTIFIC