

# Protected chambers

## For your most sensitive cells

Cell culture



The proprietary Thermo Scientific™ Cell Locker™ System, consisting of individual segregated chambers within a CO<sub>2</sub> incubator, provides enhanced culture stability and protection. The Cell Locker System divides the CO<sub>2</sub> incubator into six chambers with individual glass doors, uniquely preserving critical conditions for sensitive cells by isolating cultures from door openings.

### Designed into the following CO<sub>2</sub> incubators:

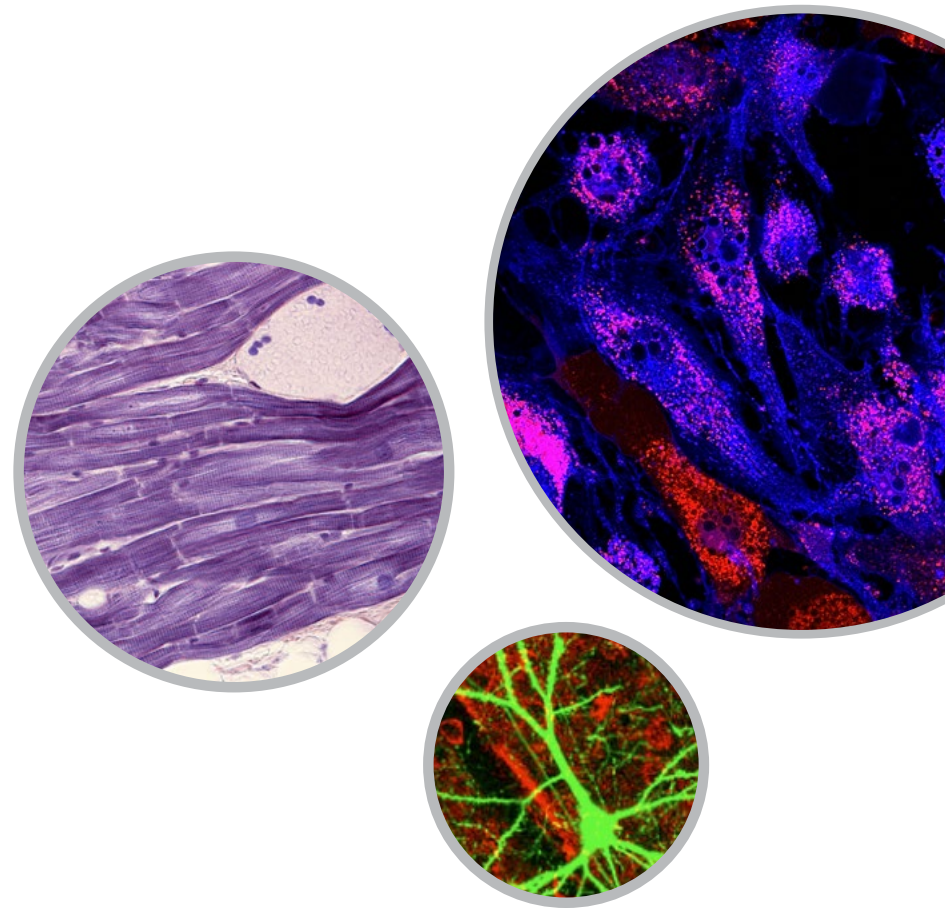
- Thermo Scientific™ Heracell™ VIOS™ 160i CO<sub>2</sub> Incubators
- Thermo Scientific™ Heracell™ Vios™ 160i CR CO<sub>2</sub> Incubators CTS™ Series
- Thermo Scientific™ Forma™ Steri-Cycle™ i160 CO<sub>2</sub> Incubators
- Thermo Scientific™ Forma™ Steri-Cycle™ i160 CR CO<sub>2</sub> Incubators CTS™ Series



## Sensitive cells like stem cells, primary cells, neural cells, and immune cells are less resilient

Changing culture conditions affect them comparatively more than immortalized cell lines. Unlike established cell lines that have been growing in culture for decades, slow-growing cell types or those that have only been passaged a few times have not had the opportunity to adapt (mutate) to artificial, two-dimensional in vitro culture conditions.

These fragile cell types generally require more exacting and labor-intensive effort to successfully culture [1]. Also, any microbial contamination is comparatively more costly if these cultures have to be discarded and the work repeated.



**The Cell Locker System is well suited for sensitive cells including stem cells, primary cells, neural cells, lymphocytes, and more.**

Protection

# Breakthrough for maintaining cell health

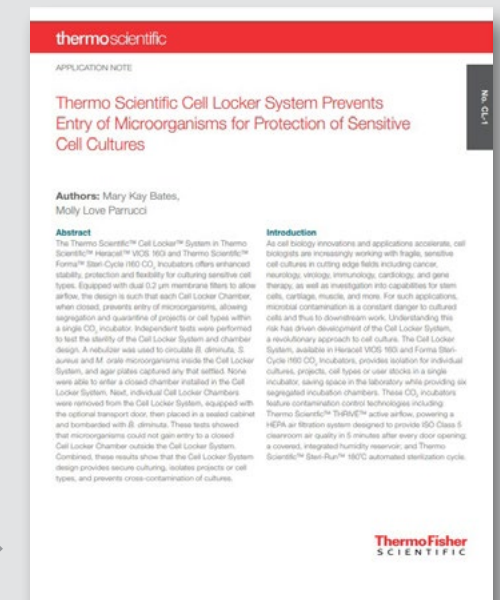
## In frequently opened or shared-use incubators

Recognizing increasing use of more fragile cell types in basic and applied research for regenerative medicine, neuroscience, immunotherapy, cell therapy, oncology, autoimmune diseases, and more, the Cell Locker System was designed to help:

- Protect these cells
- Provide a more stable environment
- Prevent cross-contamination
- Conserve atmospheric gases



**Application note: “Thermo Scientific Cell Locker System Prevents Entry of Microorganisms for Protection of Sensitive Cell Cultures”:**



Segregation

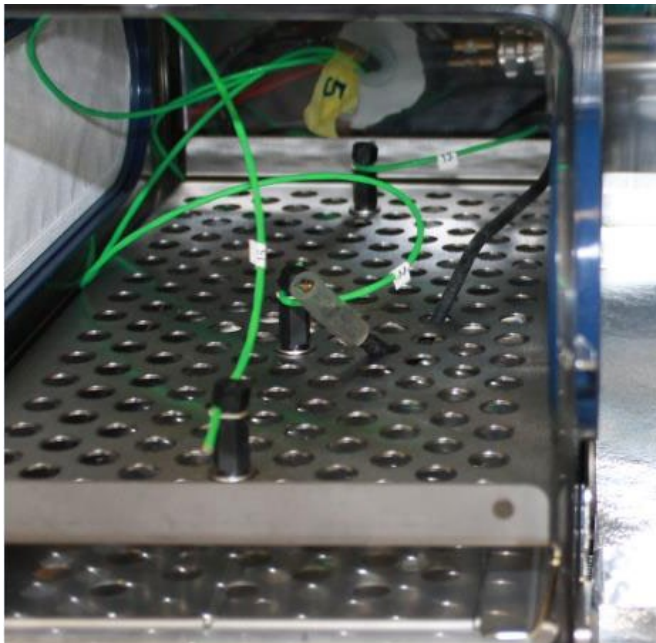
**The Cell Locker System segregates cell types, projects, or user areas**



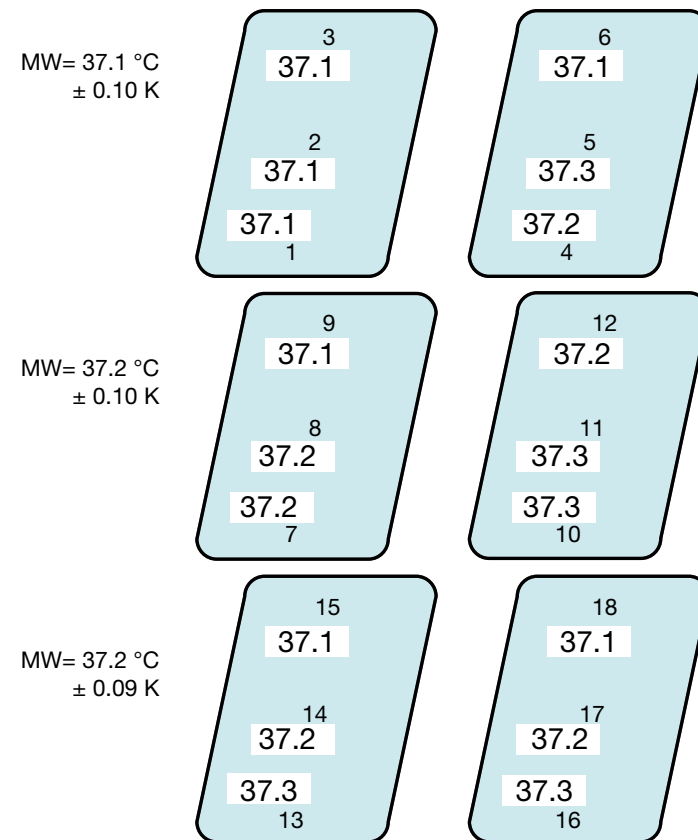
Uniformity

# All Cell Locker chambers provide a uniform environment

Thermo Scientific™ CO<sub>2</sub> incubators adhere to strict temperature uniformity throughout the incubation chamber, measured in accordance with DIN 12880. Individual Cell Locker chambers in the system meet this uniformity standard.



Temperature probes positioned according to DIN 12880 except with a distance of 10% from the side walls.



Throughout the Cell Locker System, the temperature is 37 °C ±0.3 °C.

Stability

# The Cell Locker System enhances the stability of environmental conditions

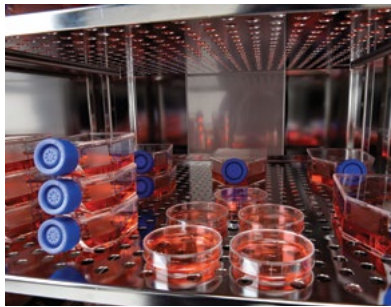
Closed Cell Locker chambers in the system do not experience the opening of a neighboring chamber.

This is very different than in a standard CO<sub>2</sub> incubator, where every door opening is experienced by all the cultured contents. When the door is opened, the incubator conditions rush to equilibrate with the ambient room conditions.



Typical laboratory

**Conditions:**  
22 °C  
0.04% CO<sub>2</sub>  
40–60% humidity



Standard Heracell VIOS 160i  
CO<sub>2</sub> incubator

**Conditions during door opening:**  
Temperature below 30 °C  
CO<sub>2</sub> below 1%  
Humidity below 30%



Closed chambers in Cell  
Locker System when one  
chamber is opened

**Maintains:**  
37 °C ±0.3 °C  
5% CO<sub>2</sub> ±0.2%  
≥93% humidity ±3%



**Application note: “Cell Locker System segregates stem cells, protecting from contamination and enhancing environmental stability”:**

thermo scientific

APPLICATION NOTE

Cell Locker System

### 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<sub>2</sub> 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<sub>2</sub> 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 resistant 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 to contamination from the air and from lab worker's normal flora, 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

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## Temperature

A stable temperature simulating body temperature native to cultured cells (in most cases 37 °C) is critical to all cultured cells, but especially for sensitive cell types.

A temperature of only 1 °C above 37 °C is detrimental and even immortalized cells will be degraded with temperatures of 2 °C above normal [2].

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## Humidity

High relative humidity (RH) is very important for sensitive cells because it prevents evaporation of water from growth media. Evaporation only involves removal of water, so the remaining salts, minerals, and other nutrients become more concentrated as a result, and can reach concentrations that are toxic for many cell types. But the Cell Locker System reduces evaporation and enables faster humidity recovery, providing improved maintenance of precisely balanced growth media.



## 3

## Carbon dioxide

Carbon dioxide works with the buffer in culture growth media to maintain stable culture pH, mimicking what happens in the vertebrate bloodstream. All cells in the body produce  $\text{CO}_2$  as a by-product of metabolism, and in the blood, bicarbonate ions are a native buffer. In the lungs, waste  $\text{CO}_2$  is approximately 5%, similar to the concentration most often used in culture, in concert with 2 mg/mL sodium bicarbonate in growth media. When pH drifts too far from physiological 7.4, cells may develop morphological stress indicators such as granules in the cytoplasm, membrane detaching and other changes [2,3].

## 4

## Oxygen

Several cell types grow faster with fewer stress indicators, less differentiation, and longer life when cultured under reduced  $O_2$ , mimicking hypoxic conditions *in vivo* [4]. In the body,  $O_2$  concentration varies from 1–4% in the brain and bone marrow to 12–14% in the bloodstream and lungs. Because  $O_2$  affects nearly every cellular process, differences in oxygen concentration can mean varied cell responses in experiments. When a standard hypoxic incubator door is opened for 30 seconds, the entire chamber rises to 15–16%  $O_2$ , approaching atmospheric oxygen of 21%. But in the Cell Locker System, when one chamber is opened, the neighboring five unopened chambers only see an increase of about 2%. So cells remain in nearly stable  $O_2$  for more uniform results.



Save gas

## Cell Locker System reduces CO<sub>2</sub> and N<sub>2</sub>/O<sub>2</sub> gas consumption by over 50%

The effect is most impactful in a hypoxic system. The Cell Locker System greatly reduces both CO<sub>2</sub> and N<sub>2</sub> loss or usage during a door opening.

If the doors are never opened, the Cell Locker System will use slightly more N<sub>2</sub> gas over time, due to additional gaskets that slowly release gas. However, in a more realistic user scenario with 30-second door openings throughout the day, the Cell Locker System uses only about half as much gas.

This means that for a typical stem cell research lab, a bottle of N<sub>2</sub> gas will last twice as long.

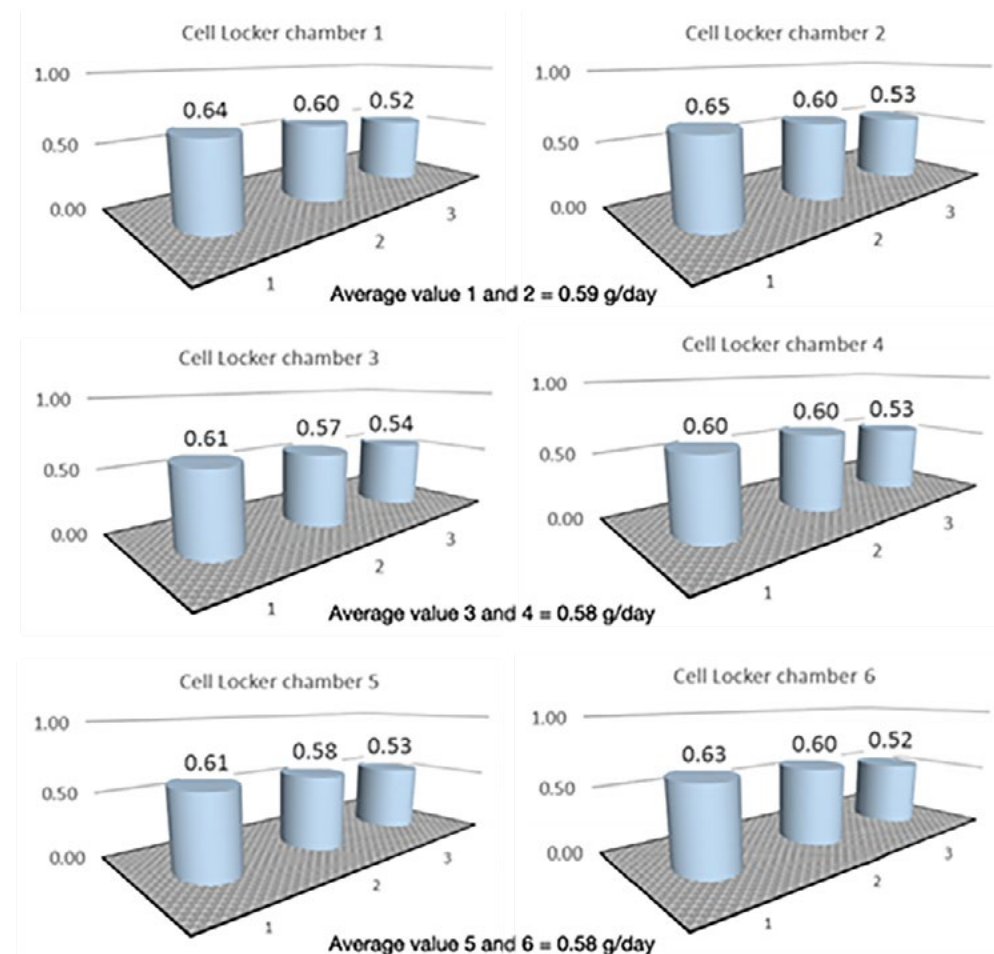
The effect is most impactful in a hypoxic system. CO<sub>2</sub> gas is also reduced by a similar percentage (results not shown).

# Evaporation is reduced by 50% in the Cell Locker System

Limiting evaporation from cell culture media is increasingly important in precise applications and is especially critical in small-volume assays such as those in microwell plates, organ-on-a-chip cultures, and microfluidic systems. When water evaporates, salts, nutrients, and other additives in growth media can reach concentrations that are toxic to cultured cells.

Thermo Scientific™ THRIVE™ airflow technology is designed for very fast humidity recovery of <10 minutes following a 30-second door opening. But the Cell Locker System provides even more stable high humidity, reducing evaporation over a 24-hour period by 50%. The average evaporation rate for the Heracell VIOS incubator is 120 g/day, while the Cell Locker System's average evaporation rate is 0.58 g/day.

**Evaporation in any Cell Locker System is an average of only 0.58 g/day.** In unopened chambers inside a Heracell VIOS 160i incubator, evaporation rates were strikingly consistent, and more than 50% lower than the rate inside the incubator itself.



# Materials and methods



# Summary

## Protected chambers for your most sensitive cells

The Cell Locker System represents an advanced 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. Compared to our CO<sub>2</sub> incubator that itself has outstanding conditions, the Cell Locker System remains more stable, recovers faster, and uses less gas even under hypoxic conditions.



### References:

1. Gibco Pluripotent stem cell guidebook (2017). Thermo Fisher Scientific COL31555 0417.
2. Freshney RI. Culture of Animal Cells (2016). 7th ed. John Wiley & Sons, Hoboken NJ.
3. Lo C-M, Keese CR, and Giaever I (1994) pH changes in pulsed CO<sub>2</sub> incubators cause periodic changes in cell morphology. Exp Cell Res. 213:391-397.
4. Bates MK (2012) Culturing cells under hypoxic conditions for biologically relevant results. American Laboratory.

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