ThermoFisher SCIENTIFIC

Cell and gene therapy

# Five reasons

## To use Cell Therapy Systems (CTS) Series $CO_2$ incubators in your cell and gene therapy production



thermo scientific

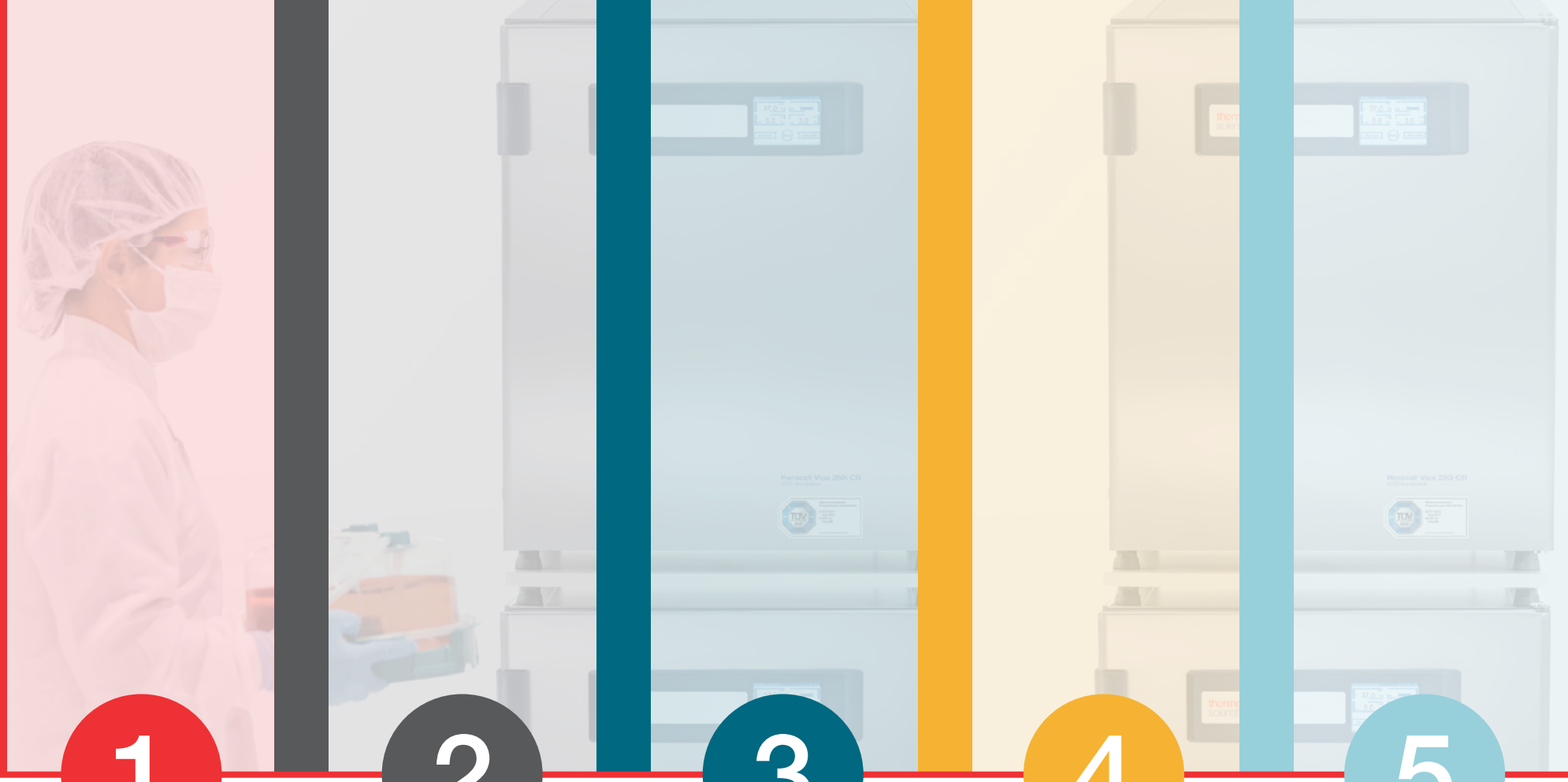
# Cell and gene therapy production

New life changing cell and gene therapies are developed every day. In moving from research to development to scale out, reproducibility of the conditions under which you developed the biologic is crucial. How the cells are cultivated can affect critical quality attributes (CQAs) including cell surface protein expression frequency, duration, potency and more. [1]

We understand that your cell culture process is unique to your new biologic, and the years you have put into development can culminate in an advancement that helps improve human health. There are so many steps in the process, so many variables to control. We can help. Thermo Scientific™ $CO_2$ Incubators Cell Therapy Systems (CTS™) Series are known the world over for their quality, performance, reliability, and reproducibility.

1 2 3 4 5

Here are **5 reasons** why you can trust these models as the center of your production process.



1 Sample protection

2 Reproducible results

3 Documentation and data capture

4 Cleaning and disinfection

5 Cost effectiveness

# 1 Proven sample protection

$CO_2$ incubators CTS series feature:

- **In-chamber HEPA filtration**
- **12-log sterility assurance level (SAL) automated heat sterilization**
- **Protected humidity reservoirs**
- **Electropolished shelving**

Together, these advanced technologies help minimize the chances of microorganisms colonizing the incubator or your cultured cells.

## In-chamber HEPA filtration

A High Energy Particulate Air (HEPA) filter will capture all particles and microorganisms regardless of size. This is due to different physical processes including impaction, diffusion and interception. [2]

Thermo Scientific $CO_2$ incubators CTS series feature in-chamber HEPA filtration which filters the entire chamber air volume every 60 seconds, reaching ISO Class 5 conditions or better in five minutes or less following every door opening. This speed of particle reduction is only possible with in-chamber components. Some incubator manufacturers locate a HEPA filter outside the incubation chamber in a by-pass loop, requiring samples of air to be pulled from the chamber and passed through tubing to the HEPA filter, driven by an air pump. This arrangement may result in incomplete air filtration and much slower time to cleanroom air quality.

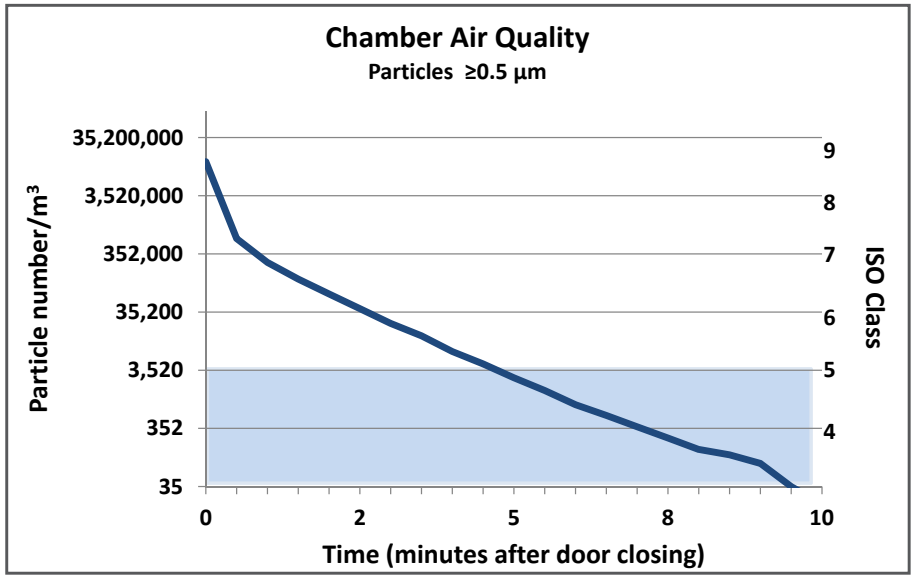



**Figure 1.** Thermo Scientific™ Heracell™ Vios™ CR $CO_2$ incubator HEPA filtration system reduces the number of circulating particulates in the incubator chamber by 10,000/$m^3$ in 5 minutes following a 30-second door opening. The entire chamber air volume is circulated through the HEPA filter every 60-seconds, with particle counts approaching zero while the incubator door remains closed.

# 1 *Continued*—Proven sample protection


## 12-log SAL automated heat sterilization

Control of microorganisms in a production cleanroom is paramount, because these unique therapies cannot undergo final filtration. Methods used for elimination must be proven effective to achieve consistent and dependable results to help protect the integrity of the product and ultimately the safety of the patient.

Sterilization is defined by the U.S. and European Pharmacopeias [3,4] as proof of elimination of one million ($10^6$) specific microorganisms that are more resistant to the treatment than any other life form. For dry heat sterilization, the accepted biological indicator organism (BI) is the endospore form of *Bacillus atrophaeus*. For autoclave sterilization, the BI is the endospore form of *Geobacillus stearothermophilus*, and specific strains with quantified heat resistance must be used. For the U.S. Pharmacopeia, a further "overkill" proof is required, where the lethal treatment is doubled, for an effective $10^{12}$ (12-log) sterility assurance level (SAL). In addition, the heated air is required to be constantly circulated "using a fan or blower" to eliminate cold spots where microorganisms could survive the treatment.

Thermo Scientific $CO_2$ incubators CTS series are unique in providing this proof of sterilization (Table 1), as well as extensive temperature maps of the incubator chambers to show that all areas reach the specified sterilization temperature and that the time at the sterilization temperature is at least double that needed to eliminate the BIs [5,6]. No other $CO_2$ incubator manufacturer meets this level of evidence.

**Table 1.** Thermo Scientific sterilization cycles are independently proven to eliminate more than 6-logs of specified biological indicators of sterilization. For each model, the sterilization cycle was tested using half the time at the specified sterilization temperature (180˚C at 45 minutes for Heracell Vios models, or 140 ˚C for one hour for Thermo Scientific™ Forma™ Steri-Cult™ $CO_2$ incubators). **This results in a 12-log SAL** for the complete cycles in production units. [5,6] **Orange** indicates the BI for dry heat sterilization, **green** indicates the BI for autoclave sterilization.

Table 1. Sterilization cycles

| Effectiveness of high temperature sterilization in $CO_2$ incubators – CTS series | | | | |
|---|---|---|---|---|
| **Incubator model** | Microorganism | Average positive control (CFU) | Number recovered | Log reduction |
| **Heracell Vios** | *Bacillus atrophaeus* spores ATCC 51189 | $2.16x10^7$ | NG | -7.3 |
| **Heracell Vios** | *Geobacillus stearothermophilus* spores ATCC 12980 | $4.81x10^6$ | NG | -6.7 |
| **Heracell Vios** | *Aspergillus brasiliensis* ATCC 16404 | $2.98x10^4$ | NG | -4.5 |
| **Heracell Vios** | *Mycoplasma pneumoniae* ATCC 15531 | $1.25x10^6$ | NG | -6.1 |
| **Forma Steri-Cult** | *Bacillus atrophaeus* spores ATCC 6633 | $4.3x10^6$ | NG | -6.6 |
| **Forma Steri-Cult** | *Aspergillus brasiliensis* ATCC 16404 | $3.13x10^6$ | NG | -6.4 |
| **Forma Steri-Cult** | *Pseudomonas aeruginosa* ATCC 15442 | $8.57x10^6$ | NG | -6.9 |

# 1 *Continued*—Proven sample protection

**Figure 2A.** Steri-Cult external reservoir can be aseptically filled without opening the incubator chamber.

## Protected humidity source

Relative humidity (RH) is commonly provided in a cell culture process to limit evaporation of culture media, maintaining precisely balanced components. Standard operating procedures (SOPs) regarding RH vary widely. When supplying water for RH in a production setting, protecting the water from microbial contamination is critically important.

$CO_2$ incubators CTS series offer different approaches to protect the water. Steri-Cult models feature an external water reservoir (Figure 2A) that can be refilled aseptically without opening the incubator chamber. The reservoir feeds an atomizer which provides controlled RH from 0% to 95% in the incubator chamber.

Heracell Vios CR and Thermo Scientific™ Forma™ Steri-Cycle™ CR models offer a simpler system. An integrated, covered water reservoir is protected by a pre-filter to help prevent larger particles and microorganisms from entering the water reservoir. As the circulating air enters the protected space, it picks up humidity and conditioned gases, then passes through an in-chamber HEPA filter to clean the air. Internal testing shows that this design helps keep the water much cleaner than a standard open water pan [7]. The cover is easily removable to fully expose the water reservoir (Figure 2B) for easy cleaning and disinfection as part of a standard GMP process [8].

**Figure 2B.** Vios CR and Steri-Cycle CR water reservoirs are fully accessible without tools for scheduled cleaning and disinfection.

# 1 *Continued*—Proven sample protection

**Figure 3.** Heracell Vios CR electropolished stainless steel incubator chamber.

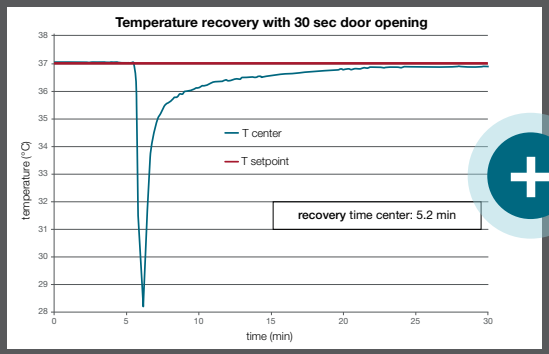
## Electropolished interior

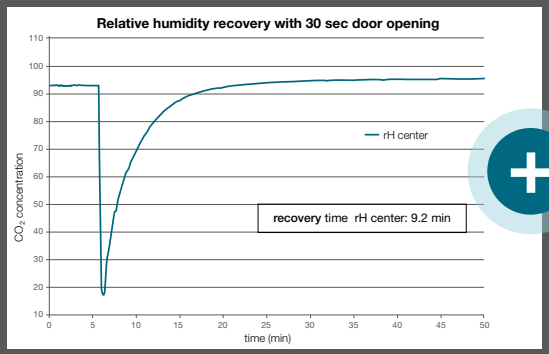
Electropolishing stainless steel improves upon mechanical polishing. The electropolishing process removes roughness and microscopic structure on stainless steel surfaces.

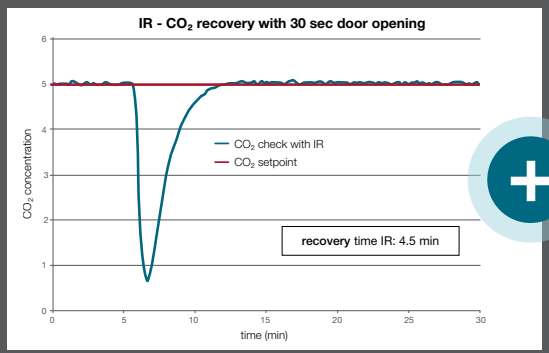
$CO_2$ incubators CTS series feature electropolished interiors (Figure 3) which reduce surface roughness (Ra) to less than 0.4 µm compared to standard Ra of less than 0.8 µm. This treatment significantly reduces small areas where microorganisms could reside, reducing bacterial attachment by up to 95% [9]. Electropolishing also improves corrosion resistance to common chemical disinfectants, which eases and speeds GMP cleaning protocols.

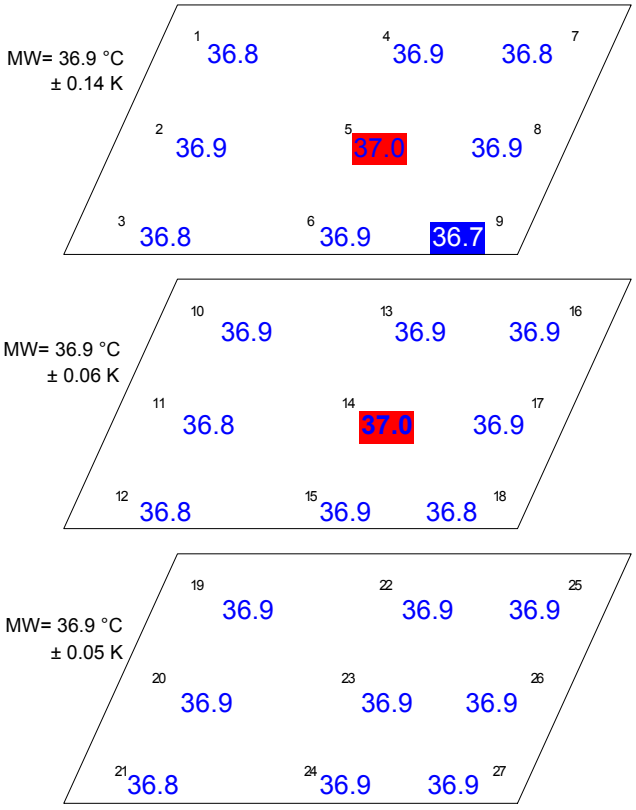
# 2 Reproducible culturing throughput

**Figure 4A.** Heracell Vios and Forma Steri-Cycle 255 L $CO_2$ incubators show recovery of all parameters in ten minutes or less from a 30-second door opening.




**Temperature unifirmity= ± 0.14 K**
Temp. fluctuation: ± 0.13 K bis ± 0.14 K (in 9h)

Specified: Temperature uniformity = ± 0.20 K max.

**Figure 4B.** A 27-point temperature map of the interior chamber in the Forma Steri-Cult model 3311 shows uniformity meets +/- 0.2 ˚C, measured according to DIN 12880.

Patient derived cells used for autologous immunotherapy or developed for allogeneic cell therapy treatments are extremely reactive. CQAs of the final cell product can vary greatly depending on the culture conditions [1]. Because these cells respond to changing or different cues, it is crucial that the conditions from top to bottom and side to side in the culturing chamber are uniform, so that all cells experience the same conditions. It is even more important that the incubator recovers quickly to desired conditions following a door opening (Figure 4A), so that cells spend their maximum time at their ideal parameters, speeding doubling time as well as helping to ensure quality. Ideal conditions for cell health and growth are not just about temperature, but $CO_2$, oxygen and RH are also important, because all play a role in cell health [10]. An incubator that ensures uniformity and fast recovery will help increase lot to lot reproducibility.

But reproducibility is not guaranteed even if one incubator has the same sensor specifications as another, because performance is more than a list of parts specifications. Look for complete temperature maps with a minimum of 27-points (Figure 4B) to provide uniformity data. Require an incubator with all sensors and probes located in the incubator chamber to ensure they measure and react to the same conditions experienced by the cultured cells. This may sound obvious, but some incubators feature a “by-pass loop” [11] with lower-quality sensors, and this information is not always clear from the manufacturer. A by-pass loop can also represent an ongoing reservoir of microbial contamination because it is difficult to sterilize.

# 3 Documentation and data capture

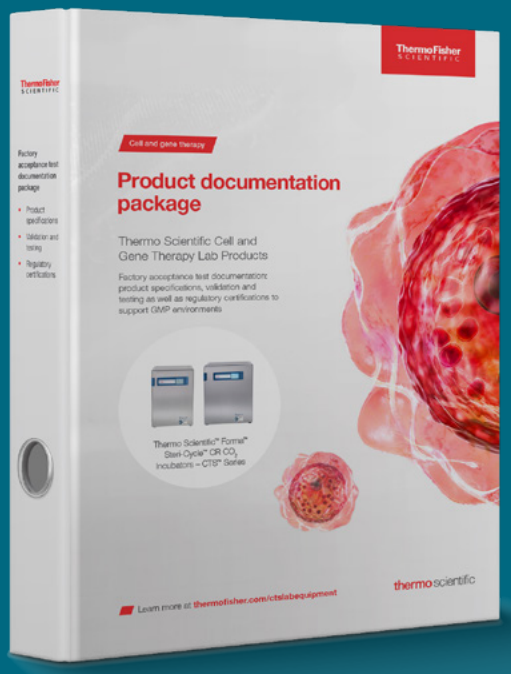



**Figure 5A.** $CO_2$ incubators CTS series are delivered with a complete documentation binder, containing everything you need to help pass your audits for these instruments.

Regulatory audits help ensure safety, efficacy, quality and purity of your cell product. Collecting and organizing all the documents for an audit requires a robust record keeping and quality management system. But this work is critical to help meet legal and regulatory requirements and to get you through audits; poor record keeping is a common flag in audit failures. Good documentation practice (GDP) for your production equipment starts with clear and complete documents including certificates, Factory Acceptance Test (FAT) data, materials of composition, manuals, technical drawings, and recommended maintenance and spare parts.

## Complete documentation package

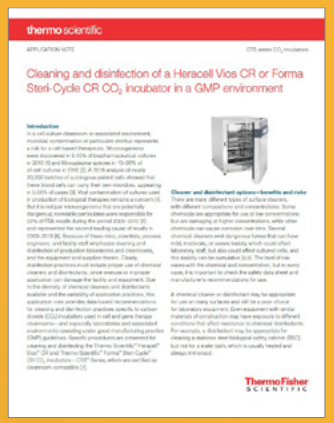
As a key part of your production process, equipment choice and acquisition is important. In addition to meeting all the documentation and quality stipulations just mentioned, the process equipment needs to be GMP compatible and meet the performance established during process development. $CO_2$ incubators CTS series are supplied with all the documentation you need to ease your regulatory audits, contained in a single hard copy binder (Figure 5A). Our FAT documentation package includes technical specifications and drawings, recommended preventive maintenance, replaceable parts lists, complete certifications, recommended cleaning and disinfection and validation protocols.

## Data capture option (OPC-UA)

$CO_2$ incubators CTS series can be integrated with OPC UA (Open Platform Communications Unified Architecture) firmware and protocol, enabling equipment communication via Distributed Control Systems (DCS). Introducing this automated process into your lab can help simplify operational complexity, lower project risk, save overall operation costs, and enables you to manage the entirety of the cell therapy workflow.


**Figure 5B.** Optional accessory to integrate Heracell Vios CR and Forma Steri-Cycle CR $CO_2$ incubators into Distributed Control Systems.

# 4 Extensive cleaning and disinfection recommendations




Read the **Application Note "Cleaning and disinfection of a Heracell Vios CR or Forma Steri-Cycle CR $CO_2$ incubator in a GMP environment"** to learn more.

Watch the **instructional video: "Cleaning and disinfection methods for a cleanroom setting"** to learn more.

When producing a cell-based biologic, the risks to the cell product from microbial and non-viable particulates cannot be underestimated. This means that for process engineers, scientists, technical staff and operations personnel, cleaning and disinfection are a way of life. But the chemicals used to help provide a clean environment also represent a risk. Overuse of the wrong chemical can cause deterioration and degradation of the surfaces and materials in any laboratory equipment. In addition, some of these chemicals produce volatile organic compounds (VOCs) which can elicit stress responses from cultured cells [12,13] that affect CQAs. Because there are so many questions about which chemicals are safe for use, we offer our own recommendations (Table 2) for common chemical products that have been tested and proven compatible as well as effective [8]. In all cases, follow the manufacturer's recommendation for dilution to a working concentration and for dwell time, and follow all chemicals with 70% ethanol or 70% isopropanol to remove residues that could otherwise build up over time, harming cultured cells and equipment.

**Table 2. Recommendations for chemicals used in manual wipe disinfection of $CO_2$ incubators.**
These disinfectants have proven compatibility with stainless steel, glass, and copper when used according to manufacturer's recommendations. 70% EtOH or 70% IPA are excellent choices for use following any other chemical, to remove residues that could cause corrosion over time. Disinfectant chemicals not listed are not recommended due to potential damage to incubator materials. For 100% copper interiors, a chemical disinfectant is not needed due to copper's natural properties. However, 70% EtOH or 70% IPA may be used without risk to the copper surface.

| Type | Concentration | Example brand |
|---|---|---|
| Ethanol | 70% | Any (common) |
| Isopropanol | 70% | Any (common) |
| Quaternary ammonium | 10% or less (2% or less is best) | Conflikt™, Lysol™ No Rinse, Fermacidal D2™ |
| Hydrogen peroxide | 1–3% | Any (common) |
| Hydrogen peroxide/peracetic acid/acetic acid | 1%/0.8%/<10% | Spor-Klenz™ Ready-to-Use (RTU) Sterilant (STERIS Life Sciences) |

# 5 Cost effective process flexibility and scale-out

**Figure 7A.** Stacked Vios CR $CO_2$ Incubator with CultiMaxx Shelving Systems, filled with up to 20x G-Rex® 500M-CS bioreactors.

With your life-changing therapeutic cell or gene therapy, you expect that regulatory approval and ongoing effective treatments will drive more demand. To better support your scale-out, we offer options for increasing vessel capacity and accessibility. These accessories can be easily retrofitted in your existing $CO_2$ incubators CTS series, helping improve your production not only in the same footprint, but in the same units.

Thermo Scientific™ CultiMaxx™ Shelving Systems are optimized for the most popular cell and gene therapy production vessels, including Wilson Wolf G-Rex® bioreactors, Thermo Scientific™ Nunc™ Cell Factory™ systems, and VueLife™ culture bags from Saint-Gobain.

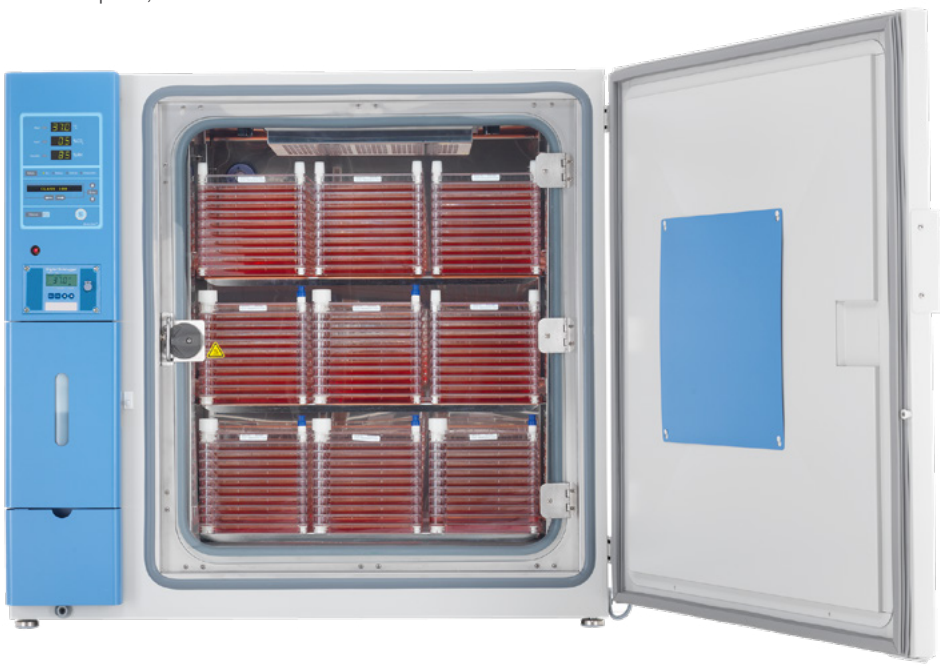
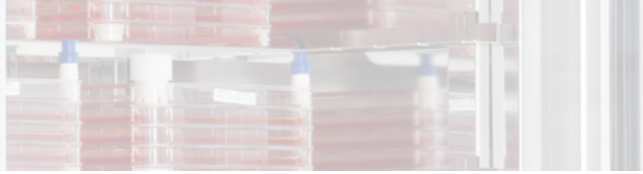


**Figure 7B**. CultiMaxx Shelving System for up to 9x Nunc Cell Factory systems with 10 or 13 layers in a Forma Steri-Cult $CO_2$ incubator.

**Figure 7C**. Cascading CultiMaxx Shelving System in a Vios CR $CO_2$ incubator with easy access to Nunc Cell Factory systems.



# Summary
## 5 reasons

Key considerations for GMP production from $CO_2$ incubators used in cell and gene therapy include proven contamination control methods like in-chamber HEPA filtration, proven sterilization, protected humidity source and an electropolished interior. CTS series models demonstrate uniformity throughout and fast recovery to desired conditions to help provide a final biologic product that meets your defined CQAs. An extensive documentation package can help support your regulatory compliance. Detailed manufacturer recommendations for cleaning and disinfection can help save you time and headaches. Compatible accessories help maximize your investment in the same footprint as your production increases.

Thermo Scientific $CO_2$ incubators CTS series are the production partners you can rely on.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