Cleanroom components
Composants pour salles blanches

- ISO 14644-1
  Class ISO **5**
- GMP-cert
  Class **A/B**

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Cleanroom setting

Five reasons

To choose a cleanroom CO_2 incubator that is certified compatible

5 A cleanroom is a special place

Depending on the air quality rating, **a cleanroom is one hundred (ISO Class 7) to ten thousand (ISO Class 5) times cleaner** than normal indoor room air (ISO Class 9). [1] A cleanroom must maintain this air quality rating to minimize contaminant risk and to assure quality production. For advanced therapy medicinal products (ATMPs), working under Good Manufacturing Practices (GMP) ensures the cellular product or gene therapy will be safe and pure, effective, and of high quality. So a production cleanroom that is ISO Class 7 or higher, or European Union classified GMP Grade A or B, helps ensure product safety and purity. Maintaining a cleanroom setting requires constant vigilance, strict adherence to GMP protocols, documentation and record keeping, and meticulous cleaning procedures.

Table 1A. ISO Classifications

ISO classification number	Maximum concentration limits (particulates/m3 of air) for particles equal to and larger than the considered sizes					
	0.1 µm	0.2 µm	0.3 µm	0.5 µm	1 µm	5 µm
ISO Class 1	10	2				
ISO Class 2	100	24	10	4		
ISO Class 3	1,000	237	102	35	8	
ISO Class 4	10,000	2,370	1,020	352	83	
ISO Class 5	100,000	23,700	10,200	3,520	832	29
ISO Class 6	1,000,000	237,000	102,000	35,200	8,320	293
ISO Class 7				352,000	83,200	2,930
ISO Class 8				3,520,000	832,000	29,300
ISO Class 9				35,200,000	8,320,000	293,000

Table 1B. EU GMP grades based on ISO 146441 [1] and EU GMP standards [2].

GMP Grade	Maximum permitted number of particles per m3 equal to or greater than the tabulated size			
	At rest		In operation	
	0.5 µm	5.0 µm	0.5 µm	5.0 µm
A	3,520	20	3,520	20
B	3,520	29	352,000	2,900
C	352,000	2,900	3,520,000	29,000
D	3,520,000	29,000	Not defined	Not defined

Here are **5 reasons** why it is important to use a certified cleanroom compatible CO_2 incubator for scale-out manufacturing of ATMPs.

1 Active Particle Control

2 Certified cleanroom compatible

3 IP54 compliant

4 VHP compatible

5 Certified accessories

1 Active Particle Control

22%

U.S. FDA recalls of sterile injectables from 2008-2012 were caused by non-viable particulates

2nd

leading cause of recalls 2009-2019

1.6 million

Moderna vaccine shots suspended in Japan in 2021

Cell therapy products represent an inherent risk to a patient due to the challenges in purification of cell products; they cannot go through a final sterilizing purification. While microbial contamination is the leading risk [4], the number two risk is from non-viable particulates. In fact, non-viable contaminants were the reason for 22% of U.S. FDA recalls of sterile injectables from 2008-2012, [5] and were the second leading cause of FDA recalls from 2009 to 2019 [4,5]. An estimated 15% of non-viable particulates come from the processing equipment used in the cleanroom [6]. As one recent example of this, in 2021, 1.6 million doses of Moderna's SARS-CoV-2 vaccine were placed on hold in Japan due to stainless steel particulates introduced due to faulty installation of processing equipment [7].

Non-viable particulates can include hair, fiber, paint, plastic, stainless steel, glass, rubber, plastic, etc. For the patient, these tiny fibers and particles can represent a range of risks from tissue damage to immune responses to blood clots to pulmonary embolism [6].

To help address all of these risks, the Thermo Scientific™ Heracell™ Vios™ CR and Forma™ Steri-Cycle™ CR CO_2 Incubators include a first-to-market, proprietary active particle control system. This design captures particles from the incubator in a HEPA filtration system on the rear wall, ensuring these particles are not released to the cleanroom [3]. The system has been tested and certified effective by an industry specialist. Use of the phrase "GMP compatible" does not equal certification, since achieving such a certification requires extensive testing [3].

Figure 1. Active particle control system in a Heracell Vios CR CO_2 incubator collects particulates from the incubator casing and directs them to a HEPA filter in the rear wall. Incoming air from the cleanroom replaces the outgoing air.

2 Certified cleanroom compatible

Figure 2A. Example of a certificate documenting compliance of a Heracell Vios 160i CR CO_2 incubator for use in ISO Class 5, EU GMP Grade A/B cleanroom. This certificate requires extensive testing, including for each individual unit produced. Thus, this certificate indicates much more rigorous testing and evidence than a label of "GMP compatible".

CERTIFICATE ◆ CERTIFICADO ◆ CERTIFICAT

TÜV SÜD Industrie Service

CERTIFICATE

TÜV SÜD Industrie Service GmbH hereby confirms

Thermo Electron LED GmbH
Robert-Bosch-Strasse 1
D-63505 Langenselbold
Germany

for the product

Heracell Vios 160i CR

the cleanroom compatibility of the equipment for the cleanroom class GMP A/ B according to EG GMP guidelines, Annex 1.

Device	Cleanroom class according to EG GMP guideline		Cleanroom class according to DIN EN ISO 14644-1	
	Particle size 0.5 µm	Particle size 5.0 µm	Particle size 0.5 µm	Particle size 5.0 µm
Heracell Vios 160i CR 37°C routine	A/ B	A/ B	5	5
Heracell Vios 160i CR steri-run decontamination routine	A/ B	A/ B	5	5

The certificate is limited to the air cleanliness by particle concentration.
The product was tested according to DIN EN ISO 14644-14 in November/ December 2020.

The implementation of the testing and certification was carried out by TÜV SÜD Industrie Service GmbH.

Certificate-no.: 3264493-00
Report-no.: 3264493-00
Valid till: December 20??

Figure 2B. Example of a sticker from an individual CO_2 incubator.

Type tested · Production monitored · Évaluation de type · Surveillée de production

TÜV SÜD

Cleanroom components
Composants pour salles blanches

- ISO 14644-1 Class ISO 5
- GMP-cert Class A/B

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Because the Heracell Vios CR and Forma Steri-Cycle CR CO_2 incubators represent a new design with a unique exterior sealed casing and a new proprietary active particle control system, we wanted to ensure they met the standards for use in an ISO Class 5, GMP Grade A/B environment. To this end, the models were tested by industry specialist TÜV SÜD Industrie Service GmbH in Germany [3]. TÜV performed extensive testing in an ISO Class 4 cleanroom, first testing the cleanability of the models inside and out, to detect whether significant levels of particulates were released during wiping. Next, TÜV tested the entire unit, identifying areas where higher particles could be released, and further testing those areas. The results demonstrate that these models are cleanroom compatible. No other incubator available today has undergone this level of testing, nor has merited this certification from TÜV. This certification and the accompanying Factory Acceptance Test package **requires extensive end of line testing of each individual CO_2 incubator** – following production, before shipment and installation – to ensure compliance and that each unit merits the certification. It is important to understand this distinction, because to say an instrument is "GMP compatible" is not the same as a certification from an independent industry specialist, and does not ensure that each unit is tested for particulate control as part of production quality assurance.

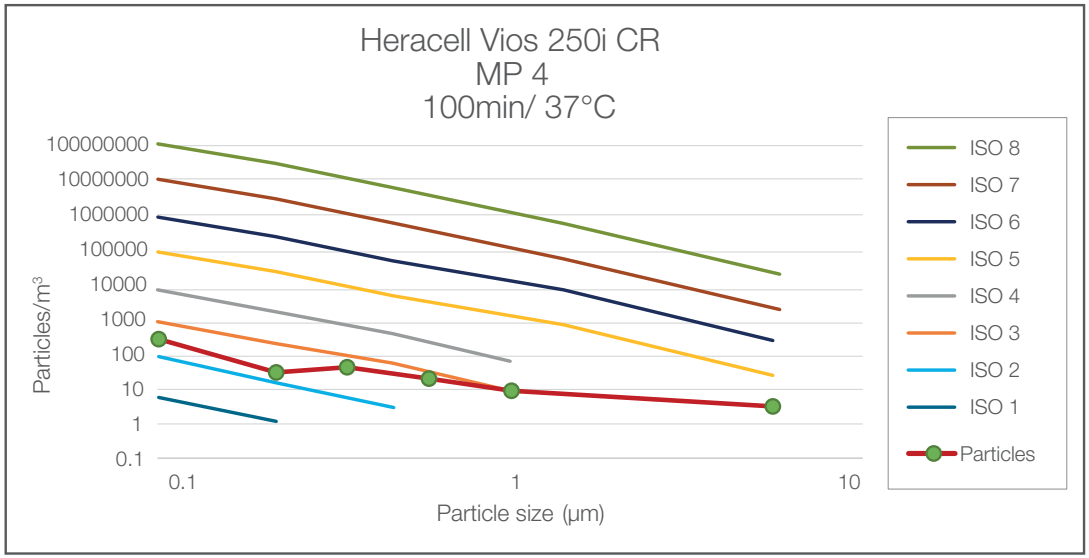


Figure 3. Example of test results from Heracell Vios 250i CR CO_2 incubator, showing particulates released fall within ISO Class 5 specifications. More information is available [3].

3 IP54 compliant

Figure 4. Heracell Vios CR – CTS series CO_2 incubators feature a brushed 304 stainless steel exterior, sealed casing and IP54 rated electronics to ensure compatibility with GMP cleaning protocols.

In a cleanroom, the rigorous processes often include spraying, wiping, and even dousing equipment with cleaners and disinfectants. For most CO_2 incubators, this can put the electronics, user interface, data logging and incubator controls at risk. For these reasons, the Heracell Vios CR and Forma Steri-Cycle CR CO_2 incubators feature a brushed 304 stainless steel exterior, fully sealed casing and ingress protection fifty-four (IP54) rated electronics. An IP54 rating means these models are resistant to ingress from dust and dirt, and from liquid sprays and spills.

This level of protection is the first of its kind for a CO_2 incubator. The certification from TÜV included evaluation of the cleanability and particulates released during this process, demonstrating the quality of the design for these incubators [3]. The IP54 rating assures that these models are an excellent match for GMP cleaning protocols. Manufacturer recommended procedures for manual cleaning and disinfection of Heracell Vios CR and Forma Steri-Cycle CR CO_2 incubators, including disinfectant recommendations, are provided as well [8].

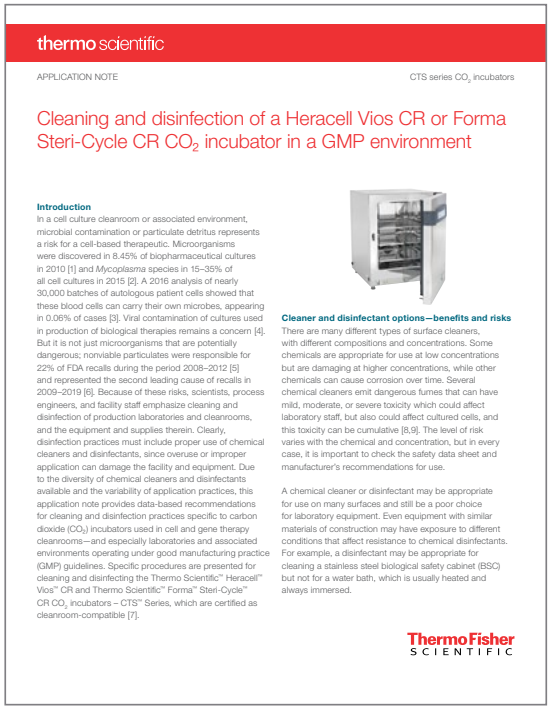
4 Proven compatibility with Vaporized Hydrogen Peroxide (VHP)

Figure 5. View of the front of a Heracell Vios 250i CR CO_2 incubator following sixteen cycles of 500 ppm STERIS VHP dry, non-condensing sterilization, showing complete compatibility with no negative effects.

Across the world, sterilization by vaporized hydrogen peroxide is now commonly used for decontamination of cleanrooms and associated equipment and instrumentation. Quite a few companies offer what is termed a "VHP" process, but not all are appropriate for laboratory equipment or instruments. Some of these vapor processes can cause pitting and corrosion of components, especially stainless steel and glass, and degradation of plastics, over time. These effects are due to lack of control of the hydrogen peroxide vapor, to the concentration used, or due to chemicals added to the hydrogen peroxide such as peracetic acid or phenol. If the process is not well managed, the vapor can condense and collect in areas of the equipment, resulting in corrosion. Also, be aware that high concentration hydrogen peroxide (35-59%) is considered highly toxic and a carcinogen [9].

For chemical sterilization of Heracell Vios CR and Forma Steri-Cycle CR CO_2 incubators, we recommend only dry, non-condensing VHP wherein the humidity is controlled to prevent condensation of the vapor because wherever any condensation occurs, corrosion can also. The VHP provider should confirm the efficacy of the procedure with evidence showing elimination of approved biological indicator microorganisms. The provider should also ensure the toxic gas is neutralized, not only for the health of the CO_2 incubator, but also for the health of your personnel and the ATMP cell cultures.

Heracell Vios CR and Forma Steri-Cycle CR CO_2 incubators were tested by STERIS Life Sciences and proven compatible with VHP. No negative effects were observed after sixteen cycles of STERIS' VAPROX® dry, non-condensing VHP procedure, each with hydrogen peroxide concentrations of 35%/ 500 parts per million (ppm) [10].



thermo scientific

APPLICATION NOTE — CTS series CO_2 incubators

Cleaning and disinfection of a Heracell Vios CR or Forma Steri-Cycle CR CO_2 incubator in a GMP environment

Introduction

In a cell culture cleanroom or associated environment, microbial contamination or particulate detritus represents a risk for a cell-based therapeutic. Microorganisms were discovered in 8.45% of biopharmaceutical cultures in 2010 [1] and *Mycoplasma* species in 15–35% of all cell cultures in 2015 [2]. A 2016 analysis of nearly 30,000 batches of autologous patient cells showed that these blood cells can carry their own microbes, appearing in 0.06% of cases [3]. Viral contamination of cultures used in production of biological therapies remains a concern [4]. But it is not just microorganisms that are potentially dangerous; nonviable particulates were responsible for 22% of FDA recalls during the period 2008–2012 [5] and represented the second leading cause of recalls in 2009–2019 [6]. Because of these risks, scientists, process engineers, and facility staff emphasize cleaning and disinfection of production laboratories and cleanrooms, and the equipment and supplies therein. Clearly, disinfection practices must include proper use of chemical cleaners and disinfectants, since overuse or improper application can damage the facility and equipment. Due to the diversity of chemical cleaners and disinfectants available and the variability of application practices, this application note provides data-based recommendations for cleaning and disinfection practices specific to carbon dioxide (CO_2) incubators used in cell and gene therapy cleanrooms—and especially laboratories and associated environments operating under good manufacturing practice (GMP) guidelines. Specific procedures are presented for cleaning and disinfecting the Thermo Scientific™ Heracell™ Vios™ CR and Thermo Scientific™ Forma™ Steri-Cycle™ CR CO_2 incubators – CTS™ Series, which are certified as cleanroom-compatible [7].

Cleaner and disinfectant options—benefits and risks

There are many different types of surface cleaners, with different compositions and concentrations. Some chemicals are appropriate for use at low concentrations but are damaging at higher concentrations, while other chemicals can cause corrosion over time. Several chemical cleaners emit dangerous fumes that can have mild, moderate, or severe toxicity which could affect laboratory staff, but also could affect cultured cells, and this toxicity can be cumulative [8,9]. The level of risk varies with the chemical and concentration, but in every case, it is important to check the safety data sheet and manufacturer's recommendations for use.

A chemical cleaner or disinfectant may be appropriate for use on many surfaces and still be a poor choice for laboratory equipment. Even equipment with similar materials of construction may have exposure to different conditions that affect resistance to chemical disinfectants. For example, a disinfectant may be appropriate for cleaning a stainless steel biological safety cabinet (BSC) but not for a water bath, which is usually heated and always immersed.

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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.

5 Certified and flexible accessories

Figure 6 A. Stacking adaptors for Heracell Vios CR and Forma Steri-Cycle CR CO_2 incubators CTS series have also been tested and certified compatible with ISO Class 5 and GMP Grade A/B cleanrooms.

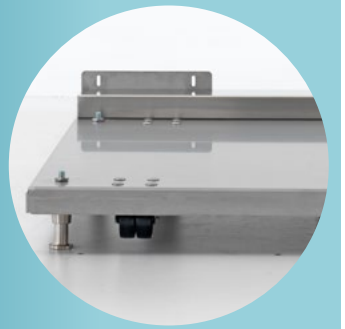

Figure 6 B. Low-profile roller base – tested and certified compatible with ISO Class 5 and GMP Grade A/B cleanrooms.

Figure 6 C. Stand – tested and certified compatible with ISO Class 5 and GMP Grade A/B cleanrooms.

Heracell Vios CR and Forma Steri-Cycle CR CO_2 incubators are ready to scale with you as you progress from development to production of your ATMP. They are available in two sizes (165 L and 255 L) and are stackable to help maximize production per footprint in the cleanroom. The accessories are also tested and certified cleanroom compatible [3], including stand, roller base and stacking adaptor. As with the incubators themselves, the materials of composition, specifications and compatibility certificates are all included in the documentation package provided with each incubator [11]. Note that marking a CO_2 incubator "GMP compatible", or even certified, does not necessarily include the accessories; it's important to understand what you are getting to avoid hidden costs later, because some accessories are made of composites that will shed particles over time.

Figure 7. Overview of a scale-out cleanroom featuring Heracell Vios CR CO_2 incubators Cell Therapy Systems Series and related CTS Series laboratory equipment.

Summary
5 reasons

When considering a CO_2 incubator for scale-out production of ATMPs including cell therapy, think carefully about what you are buying. To say that an incubator is "GMP compatible" is not equal to a certification for use in an ISO Class 5 and GMP Grade A/B environment. A certification is granted by an industry specialist in accordance with ISO 14644 and based on design and individual unit tests to ensure compatibility with these cleanroom standards. This compatibility encompasses tests of particle release during manual wiping and disinfection, and during normal use as well as during automated sterilization. Cleanroom compatibility also requires proven resistance to common disinfectant chemicals including VHP® and manual cleaning protocols. Important in these processes are IP54 rated electronics, brushed 304 stainless steel exterior, and manufacturer recommended protocols for cleaning and disinfection. Accessories should also be tested and certified compatible. To help ensure success when manufacturing ATMPs, look for independent tests of manufacturer claims and choose a CO_2 incubator that is clearly certified to meet the high standards required.

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Learn more at **thermofisher.com/cleanroomco2**