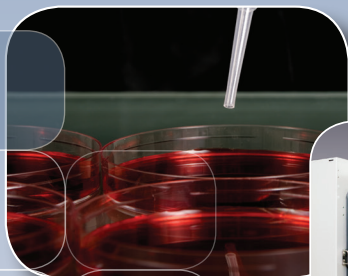


Selection Criteria and Important Factors to Consider in Choosing the Correct CO₂ Incubator for your Cell Culturing and Cell Research in your Laboratory



INTRODUCTION

CO₂ Incubators are one of the most widely used items of laboratory equipment in any typical cell culturing laboratory environment. The CO₂ incubator is therefore an essential item of equipment in the typical research and clinical laboratory environment and commonly used for the following applications: cell culturing, tissue engineering, neuroscience, cancer research, in-vitro fertilisation, embryonic stem cell research, stem cell research and other mammalian cell research and cultivation of mammalian cells. The CO₂ incubator is a very important piece of laboratory equipment to cultivate your cells. The perfect in vivo environment (conditions required inside your CO₂ Incubator inner chamber); that is most commonly used for cell research and cell cultivation is typically: 37.0°C (degrees Centigrade), 5.0% CO₂ (carbon dioxide) and 85-90% RH (relative humidity).

It is therefore very important to take your time in your selection criteria before purchasing your CO₂ Incubator and to consider some very important and critical factors (please refer to Important Factors listed on Page 2).

2

Esco Manufacturing Facilities

Esco a world class, global leading manufacturer with 35 years of experience manufacturing laboratory equipment, clean air equipment and laboratory isolators; manufactures the most user friendly and accurate CO₂ Incubator available on the market today.

Esco manufactures CO₂ Incubators that offer precise Temperature, CO₂ and Humidity conditions for your critical cell research.



IMPORTANT FACTORS TO CONSIDER BEFORE CHOOSING WHICH BRAND OF CO₂ INCUBATOR TO PURCHASE

Some of the most Important Factors to seriously consider during your selection criteria for a CO₂ Incubator for your laboratory are as follows:

Types of Heating Profile to Consider for Your CO₂ Incubator:

Direct Heat Air Jacket vs Water Jacket

Critical Factors: uniformity and control, fastest CO₂, humidity and temperature recovery without overshoot, heating profile to achieve fastest recover with minimal temperature fluctuations.

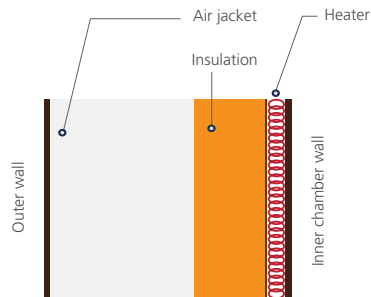
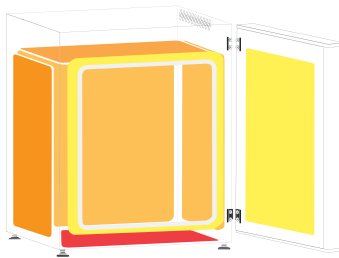
Esco manufactures direct heat combined with an air jacket to achieve the most stable homogenous and accurate chamber conditions for culturing cells.

This type of incubator is easier to set up, and will get to its set temperature for the first time more quickly because there is a great volume of air movement. It is also easier (less heavy) to move and will have faster temperature recovery time.

On the other hand, using a water jacketed incubator includes longer time for the initial set up (since it takes awhile to fill the jacket with water and get the water up to temperature for the first use) and they are also heavier, so more cumbersome to move. User also must drain the water before moving the unit and this can be very time consuming and the incubator would lose its structural strength if the water were left in.

3

DIRECT HEAT AND AIR JACKET



- The main heater provides precise temperature control.
- The bottom heater warms the water pan and controls humidity.
- The outer door heater prevents condensation on glass door and facilitates temperature recovery.

- Direct heating enables rapid temperature recovery while air jacket provides isolation against ambient temperature fluctuations.
- Precise heating in the chamber is achieved by using 8 heaters (3 zones). The 3 zones are intelligently controlled by the microprocessor for best temperature minimal fluctuation.

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Types of Carbon Dioxide (CO₂) Sensor Technology:

a. Thermal Conductivity (T.C.) CO₂ Sensor

- Usually the more economic CO₂ Incubator is fitted with a T.C. CO₂ Sensor. The main drawback of T.C. is that the unit must be at the correct percentage of Relative Humidity (%RH) for accurate measurement of CO₂. After each inner glass door opening and closure there is a considerable *delay period of time before the %RH recovers back to typical 90.0%RH.

*The delay of %RH recovery is dependent on factors such as: how long the inner glass door was opened, size of inner chamber and number of inner glass door compartments fitted.

b. Infra-Red (I.R.) CO₂ Sensor

- Is the more accurate CO₂ sensor for measuring the concentration of CO₂ inside an incubator inner chamber.

Measures accurately, regardless of the actual % of Relative Humidity inside the inner chamber. Esco fits as standard a very high quality Drift Free Infra-Red CO₂ Sensor across the Esco 50, 170 & 240 litres Celculture CO₂ range.

4

Esco IR Sensor

- An IR sensor is a versatile instrument for measuring CO₂ level inside the incubator. The CARBOCAP® sensor is silicon based and its operation is based on the NDIR Single-Beam Dual-Wavelength principle.

IR based sensors are not affected by water vapour, dust or most chemicals. The single-beam dual wavelength technology (one reference and one measurement) ensures a Drift-Free sensor that does not require calibration by the user.



Types of Convection System:

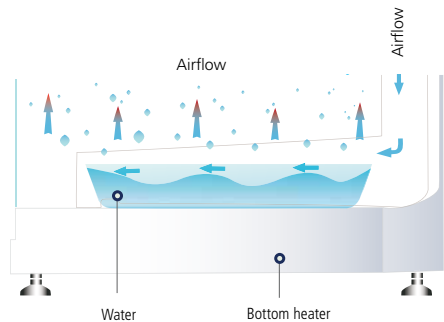
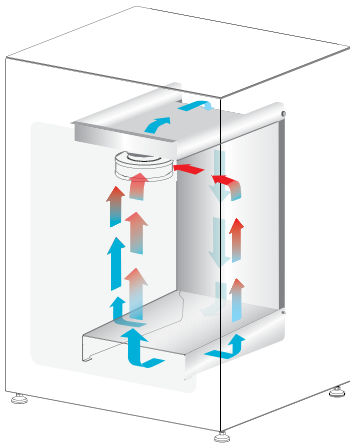
a. Forced Convection (Gentle Air Movement by a Fitted Fan)

- Considered a much better choice to minimise critically demanded recovery times of Temperature, CO₂ and % RH after each inner glass door opening. Esco cleverly directs the air; by creating a very gentle air movement through the plenum and through the ULPA Filter to achieve Class 5 air quality inside the inner chamber (Class 5 is equivalent to a very low 100 ppm of air particles).

Esco VentiFlow™ Forced Convection

- No disturbance to cell culture.
- Blower automatically stops when door is opened to minimize mixing of chamber and room air.
- Accelerates recovery of chamber air to ISO Class 5 Cleanliness after door closing to prevent contamination.
- Improves CO₂, humidity and temperature uniformity, homogeneity and control.
- Filtered air circulates across water pan to accelerate humidifying process.

5



b. Natural Convection (No Internal Fan)

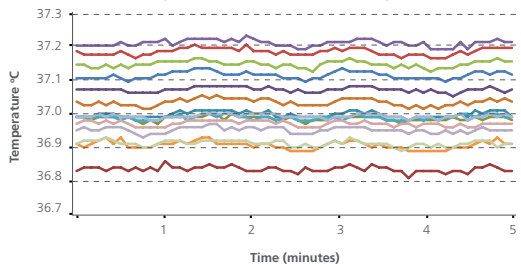
- The Benefit is absolutely no vibration, but recovery times are typically much longer and the homogeneity and stability are not as accurate as the Esco CO₂ Incubator.

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Recovery Rates and Accuracy of Recovery to Programmed Set Point Parameters After Each Inner Glass Door Opening:

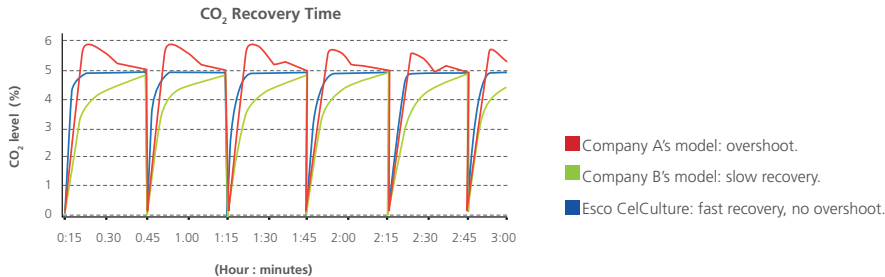
Esco VivoCell™ Precise Parameter Control Best Uniformity and Control Among Competition



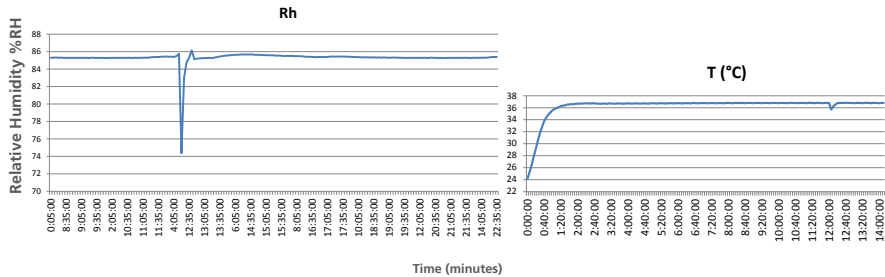
Different lines represent different sensor positions inside the chamber. Esco CelCulture has uniformity variance of less than ± 0.2 which means all the samples are evenly heated.*

6

Fast CO₂, Temperature and Humidity Recovery Without Overshoot



Precisely tuned sensor and software results in faster recovery of CO₂ without overshoot. This ensures uniform CO₂ levels even with frequent incubator door openings.



*Similarly, temperature and humidity recoveries are twice as fast as conventional incubators.

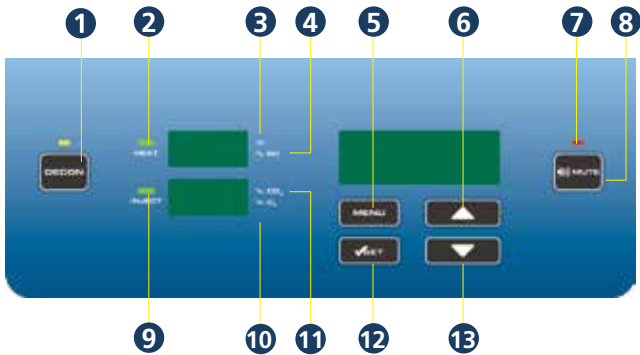
* Units were factory tested under controlled environmental conditions per Esco method. Esco does not guarantee identical results in the field under differing conditions. Original report available upon request. Model used in the test is CCL-170B-8.

Ease of Programming and User Friendly Control System and Built In Data-Logging:

In your selection process it is important to carefully consider just how user friendly are the programming menus and other control parameters on the CO₂ Incubator? Also take into consideration if the CO₂ Incubator has on board diagnostic and help menus and data-logging. Validation is now becoming more and more demanded in the research laboratory environment. Considering this in advance before purchasing your CO₂ Incubator may prove to be very prudent.

All Esco CO₂ CelCulture models come as standard with built in Data-Logging, Diagnostic and Help Menus.

All Esco CO₂ Incubators are simple to programme and navigate to other menus.



7

- | | |
|---|---|
| 1. Start / stop decontamination cycle | 8. Mute alarms |
| 2. HEAT LED Lights when heat is applied to chamber | 9. INJECT LED Lights when gas is injected |
| 3. °C is lit when displaying the temperature | 10. %O ₂ is lit when displaying the O ₂ concentration |
| 4. %RH is lit when displaying the humidity level | 11. %CO ₂ is lit when displaying the CO ₂ concentration |
| 5. Enter menu / go back to previous menu | 12. Confirm value / enter a menu |
| 6. Scroll up / increase value | 13. Scroll down / decrease value |
| 7. ALARMS LED Will blink when errors and warnings occur | |

User Friendly Software Interface:

Typically the common interface options are: RS232, RS 485, USB, SD Card and Ethernet. These are some of the various options available on the market today.

It is also important to consider how to and the best means of data retrieval, downloading onto a software suite package.

Esco offers a very user friendly Voyager Software for continuous logging of incubator chamber Temperature, %CO₂, %RH, (and %O₂ if option is fitted).

- Ability to generate reports and graph of different device parameters

- Protect the samples by providing alarms when exceeding a user-defined parameter limit via automatic email alerts



Compatible Esco Equipment (Clockwise from top):

Lexicon Ultra-low Temperature Freezer (UUS)

Isotherm Refrigerated Incubator (IFC)

Isotherm Forced Convection Incubator (IFA)

Isotherm Forced Convection Oven (OFA)

CelCulture CO₂ Incubator (CCL)

Quality and Cleanliness of Atmosphere inside your CO₂ Incubator:

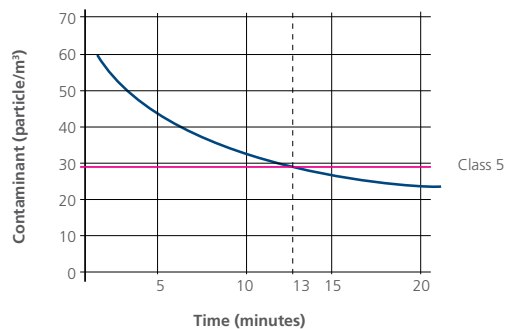
It is very important to consider how to prevent the risk of contamination and cross contamination between samples when culturing inside your CO₂ incubator. Many global CO₂ Incubator manufacturers, offer no means to ensure a cleansed air quality inside the inner chamber; and thus any samples that may be exposed inside the inner chamber may well be subjected to a possible contamination risk. Manufacturers of CO₂ Incubators with no internal fan cannot possibly fit either a HEPA or ULPA filter, so it is important to consider how clean you wish your inner chamber air quality to be, before purchasing a CO₂ Incubator.

A few manufacturers that fit an internal fan motor do fit a High Efficiency Particulate Air (HEPA) filter, removing 99.97% of all particles greater than 0.3 microns from the air that passes through.

Esco does not consider the HEPA to provide adequate enough protection as demanded by today's cell researchers and scientists and today's very important research. Therefore, Esco has deliberately gone a considerable step further by becoming a global leader in CO₂ Incubator manufacturing by fitting an ULPA filter as standard.

Esco offers a built in Ultra Low Penetration (ULPA) Filter to help minimise any air borne spores and bacteria inside the incubator inner chamber and to cleanse the air inside the incubator to a very impressive I.S.O. Class 5 category - 99.999% efficiency.

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Types of Sterilization / Decontamination Cycle to Consider for your CO₂ Incubator:

It is very important between research or cell culturing batch runs that the unit is thoroughly cleaned to minimise risk of possible cross contamination in the chamber or between the cell cultures in the petri dishes, multi-well plates, flasks or bottles.

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Typical Sterilization and Decontamination Cycles are as Follows:

a. Moist Heat / Wet Sterilization

- Proven to be 100% effective against All microorganisms, please refer to table on page4, Esco offers this Sterilization solution across its range of CO₂ Incubators.

b. Dry Sterilization

- Only effective if high enough temperature typically above 160°C is achieved and typically for greater than four hours. Some of the more robust microorganisms such as bacillus spores can survive a dry heat cycle.

c. High Temperature Decontamination

- Usually not so effective; manufactures offer cycles of only 120°C to 145°C dry heat (cannot be classified as sterilization temperature) and quite a few microorganisms can survive such high temperature decontamination cycles.

d. Ultra Violet Light (U.V.)

- Only effective and successful if all surfaces including shelves are fully exposed to the U.V. The main problem is it is almost impossible to expose All Surfaces to the U.V. there will always be hidden crevices and in particular underside of shelves that are not fully exposed to the U.V. light (areas of shadowing), giving a higher risk of some microorganisms and contamination surviving.

e. Hydrogen Peroxide (H₂O₂)

- Very effective, but must be performed in a completely closed and isolated environment. Can carry the health risk of possible exposure to the end users if for some reason the Hydrogen Peroxide is not fully neutralised back to water by the U.V. during the final stage of the cycle. Carefully conduct a risk assessment based on number of units, size of your laboratory and ventilation systems before purchasing CO₂ Incubators with a hydrogen peroxide decontamination cycle.

f. No sterilization or decontamination

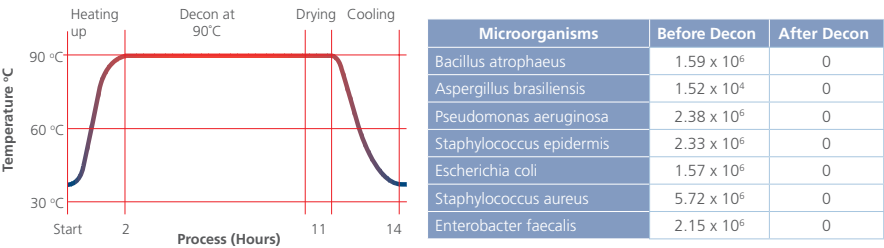
- If no cycle is fitted (usually the case on cheaper entry level CO₂ Incubators), then it is mandatory to routinely clean the inner chamber, shelves, shelf locators, any ducting and plenum, fan blade etc... by hand using a sterilise cloth and usually a 70% alcohol and distilled water solution. Unavoidable, this type of CO₂ incubator means a significant loss of culturing time over each year and it is highly recommended that this is considered before agreeing to purchase such a cheaper CO₂ incubator without any sterilization or decontamination cycle.

A regular cleaning protocol will also need to be considered by the laboratory principle / laboratory manager.

The above list illustrates many different types of sterilising and decontamination processes offered by various manufacturers of CO₂ Incubators.

Esco believes the best option to consider is a Moist Heat Sterilization Cycle at 90.0°C, proven to be 100% effective against all Microorganisms typically associated to be present inside a CO₂ Incubator inner chamber:

VALIDATED SWIFTCON™ OVERNIGHT DECONTAMINATION CYCLE



Built, Performance and Quality of Your CO₂ Incubator:

Built

All Esco manufactured CO₂ Incubators are subjected to the most stringent Quality, Testing and Calibration Protocol.

Esco believes it is extremely important to and commits to intensive testing of every single CO₂ Incubator Esco manufactures. All new Esco CO₂ Incubator are provided with all the necessary installation tools and gas tubing, securing clips, a detailed Operators Manual and the full Factory Test Report Data, including ALL checks undertaken and offsets entered.

Esco ensures that each CO₂ Incubator manufactured is of the highest standards and build quality.

Performance

Esco is very confident that the Esco range of CelCulture CO₂ Incubators, is one of the most accurate in terms of homogeneity, uniformity and control available on today's market.

Quality

Esco manufacturing standards is to ISO 9001 and ISO 14001.

Esco can also offer a full protocol booklet for on-site, sited IQ/OQ validation as demanded by many pharmaceutical organisations.

Directly Stackable without Extra Costs Associated with Stacking Kits and Stacking Frames:

A number of CO₂ manufacturers charge the customer for purpose built stacking frames / stacking kits.

All Esco CelCulture CO₂'s are directly stackable. Each unit is supplied with stacking brackets to secure a second unit that can be easily stacked on top of the first unit. Each unit is designed to take the load of another unit. The bracket and fastening bolts and instructions are included in the packaging of each CO₂ Incubator.

Any Anti-microbial Isocide paint on your CO2 incubator's external surfaces:

It is important to consider the quality of the exterior finish of your CO₂ incubator.

Does it have any external anti-microbial coating added to the exterior finish to eliminate surface bacteria that may try to survive and grow on the external surface(s) of your CO₂ Incubator.

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Esco CO₂ incubators have ISOCIDE, an antimicrobial inhibitor that eliminates 99.9% surface bacteria within 24 hours of exposure. It is integrated into the exterior coating and cannot be washed out or diminished by repeated cleaning.

Options:

It is important to consider the options you may need for your CO₂ Incubator.

Esco has a very comprehensive range of options, some examples:

a. Oxygen Control 1.0-20.7% O₂ Option:

Esco offers depleted O₂ (Oxygen) option 1.0% - 20.7% O₂ on ALL CO₂ models 50, 170 and 240 litres CO₂ Incubators.

In certain circumstances and in typical applications such as cultivation of Primary Cell Lines and in addition to the typical 5.0% CO₂ requirement; depleted O₂ levels are also required inside the chamber of the incubator:

- Arterial Blood 10% O₂
- Tumor 5% O₂
- Liver 5%-7% O₂
- Eye 10% O₂
- Lower Lung Tissue 15% O₂
- Stem Cells 5% O₂
- In Vitro Fertilisation 5% O₂
- Cancer Research

Esco O₂ Sensor:

Esco's O₂ sensor is a unique galvanic cell type oxygen sensor. Its most notable features are long life expectancy, excellent chemical durability, and it is not influenced by concentration of CO₂ inside the inner chamber of the incubator.

b. Multi-inner Glass Door Options:

It is advisable to consider your gas consumption, loss of chamber conditions and recovery times. Fitting a multi-inner glass door option will help to minimise loss of chamber atmosphere on a single compartmental door opening and therefore significantly improve recovery time.

This is a particularly important factor to consider if you have the 1.0 – 20.7% O₂ option fitted to your CO₂ Incubator.

Example: Nitrogen N₂ is purged into the inner chamber after each door opening to achieve your depleted O₂ set point, typically 5.0% O₂ in many circumstances. Nitrogen gas is quite an expensive gas, so minimising loss of N₂ over a period of time makes good commercial sense and the multi-inner glass door option will help to save costs of N₂ gas.

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c. Copper Inner Chambers:

Some researchers prefer to use optional 100% pure copper for inner chambers.

Copper has been known for millennia to have anti-microbial properties.

Esco offers an optional 100% pure copper interiors to provide additional protection for your precious samples.



Testing & Certification



For IVF applications, Esco CelCulture CO₂ incubators are certified EMBRYO-SAFE.

Rigorously tested with the Mouse Embryo Assay (MEA), the CelCulture remarkably has 100% embryo survival. The Mouse Embryo Assay (MEA) is the de facto standard test done to demonstrate that a procedure or an article of equipment is safe to use for manipulating human embryos (e.g., in vitro fertilization or IVF).

The Esco CelCulture CO₂ incubators is listed by Underwriters Laboratory (UL)*, to meet the requirements of both the U.S. and Canada standards for electrical/mechanical integrity.

**applicable for 170L*

HPA Validated Decontamination Cycle

The Esco CelCulture CO₂ Incubator 90°C decontamination cycle has been evaluated and shown to be an effective method for deactivation of the normally resistant fungi and bacterial spores *Aspergillus brasiliensis* and *Bacillus atrophaeus*, and the vegetative cells *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterobacter faecalis* and *Escherichia Coli*.

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SUMMARY

In summary Esco has undertaken lots of market research, analysed many competitors CO₂ Incubators, listened to our customers and made every effort to produce the best quality CO₂ Incubator range on today's market. We wish you every success in your CO₂ Incubator selection and trust you will consider the Esco CO₂ Incubator CelCulture range in your selection process.

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The Esco Group of Companies is a global life sciences tools provider with sales in over 100 countries. The group is active in lab equipment, pharma equipment and medical devices. Manufacturing facilities are located in Asia and Europe. R&D is conducted worldwide spanning the US, Europe and Asia. Sales, service and marketing subsidiaries are located in 12 major markets including the US, UK, Singapore, Japan, China and India. Regional distribution centers are located in the US, UK, and Singapore.

Life Science • Chemical Research • Assisted Reproductive Technology (ART) • Pharmaceutical Equipment • General Equipment

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