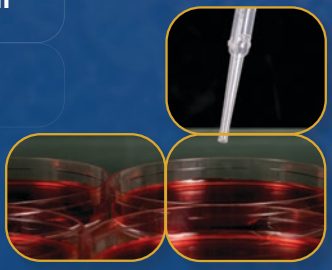
Selection Criteria and Important Factors to Consider in Choosing the Right CO_2 Incubator for your Cell Culture Laboratory



ESCO SCIENTIFIC

Table of Contents

Welcome to Esco Lifesciences

Esco Lifesciences' vision is to provide enabling technologies for scientific discoveries to make human lives healthier and safer.

Esco Lifesciences is committed to delivering innovative solutions for the clinical, life sciences, research, industrial, laboratory, pharmaceutical, and IVF communities. With the most extensive product line in the industry, Esco has passed a number of international standards and certifications. Esco Lifesciences represents innovation and forward-thinking designs, that are of the highest standard quality since 1978.

Availability and Accessibility. Esco Lifesciences has headquarters in Singapore, Indonesia, and Philippines, with manufacturing facilities located in Asia and Europe. Research and Development (R&D) is conducted worldwide spanning the US, Europe and Asia. Sales, services, and marketing subsidiaries are located in 42 major markets including US, UK, Japan, China and India. Esco regional distribution centers are located in Singapore, Malaysia, Thailand, Vietnam, Myanmar, Indonesia, Philippines, Bangladesh, Hong Kong, Taiwan, South Korea, China, Japan, India, UAE, Central and South Africa, Denmark, Germany, Italy, Lithuania, Russia, United Kingdom, and USA. Because of our worldwide presence, you can be sure that Esco is within your reach.

High Quality, Reliable, and Dependable. Esco Lifesciences products are of high quality, reliable, and dependable. Cross-functional teams from Esco Production, R&D, Quality Assurance, and Senior Management, are regularly assembled to review and implement areas for improvement.

Esco Lifesciences Cares for Your Safety. Esco Lifesciences focuses on providing safety not just for your samples, but also for you and the environment.

Esco Lifesciences Cares for Your Comfort. Building ergonomic designs and reducing noise levels of the units ensure comfort for our users.

Esco Lifesciences Cares for the Environment. Esco Lifesciences incorporates the latest proven technologically advanced components available. One in every four of Esco's employees is involved in Research and Development and are evaluating new components or designs for better efficiency. Whenever a new technology is available, Esco Lifesciences redesigns technology into our new products that will use lesser energy.

Customer Service and Support. Our service does not stop once purchase has been done. Esco Lifesciences gives on-time customer service such as service training, preventive maintenance, and re-certification, to respond to your equipment needs. Esco Lifesciences also offers free end-user seminars and provides educational materials and informative videos.

As Esco Lifesciences takes the opportunity to respond to the world's needs, we aim not only to contribute to the advancement of scientific discoveries but also in making the world a safer, healthier, and better place to live in.

I. INTRODUCTION

IMPORTANCE OF A CO_2 INCUBATOR

A CO_2 incubator is a device designed to emulate the cells' natural environment by controlling physical parameters such as the temperature, humidity, CO_2, and O_2 levels for the optimum growth and development of cells. It provides a high-quality incubation performance through the precise control of heating and gas injections combined with multiple contamination control and ergonomic features appropriate for the safe incubation of many types of cells and tissues.

Research and clinical laboratories use this equipment for applications such as in neuroscience, stem cell and cancer researches, *in vitro* fertilization, tissue engineering, and other mammalian cell research.

II. ESSENTIAL FACTORS TO CONSIDER WHEN CHOOSING THE RIGHT CO_2 INCUBATOR

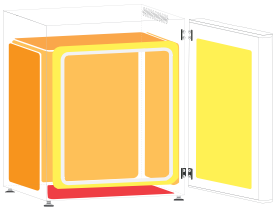
A. Types of CO_2 Incubator

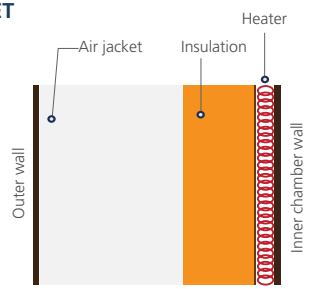
There are two basic types of CO_2 incubators being offered in the market— Direct Heat and Water Jacket. Both of these technologies provide temperature uniformity within the CO_2 incubator's chamber for optimal growth of cells.

Water-Jacketed CO_2 incubators maintain temperature by surrounding the chamber with hot walls generated from the heated water. The heated water circulates and radiates heat around the inner chamber which maintains constant temperature. However, this type of equipment takes a longer process when filling and heating the chamber. Once the chamber is filled with water, they tend to be heavy and can be difficult to transfer. The stagnant warm water being used is a critical factor in this incubator as this may cause contaminants to grow. Also, the chamber may rust if the wrong type of water is used during operation, leading to costly repairs.

Esco manufactures a direct heat combined with an air jacket CO_2 incubator to achieve the most stable and accurate chamber for culturing cells. This type of CO_2 incubator is easier to set up, and will get to set the temperature faster because there is a great volume of air movement. It is also easier to move and will have a faster temperature recovery time.

DIRECT HEAT AND AIR JACKET





- 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 minimal temperature fluctuation.

B. Types of Carbon Dioxide (CO_2) Sensor Technology

a. Thermal Conductivity (TC) CO_2 Sensor

TC sensor operates by measuring the resistance of CO_2 versus the ambient air (reference gas) and detecting changes in the resistance of the gas based on the CO_2 input flow. TC sensor is readily affected by humidity and temperature since these two factors affect air resistance. Therefore, opening the door of the CO_2 incubator easily affects the sensor, leading to inaccurate measurements.

b. Infrared (IR) CO_2 Sensor

IR CO_2 sensor operates on the theory that light is absorbed by gases at specific frequencies. The sensor measures the IR light directed at the CO_2 gas, while other wavelengths are prevented from hitting the sensor via a filter. Higher CO_2 levels would cause fewer IR rays to pass through the filter and be detected by the sensor. Low CO_2 levels would cause more IR rays to be detected by the sensor. This sensor provides accurate and stable measurements in varying conditions.

Esco offers state-of-the-art CO_2 incubator models equipped with IR CO_2 sensor. This sensor is equipped with advanced sensor technology for long-term stability, not affected by high-temperature and humidity.

GMP 251 CO_2 Probe

CCL-170_-_

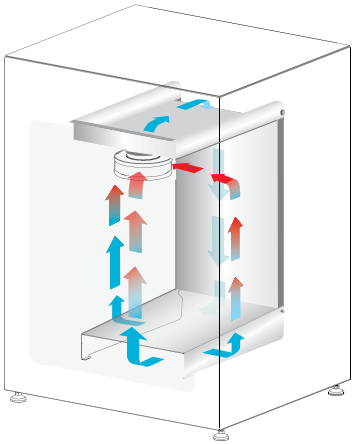
C. Types of Convection System

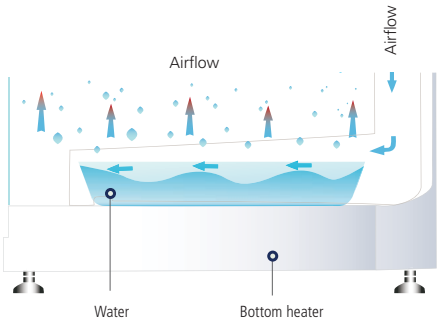
a. Forced Convection (Gentle Air Movement by a Fitted Fan)

Considered a much better choice to minimize critically demanded recovery time of temperature, CO_2, and % relative humidity after each inner glass door opening. Esco CO_2 incubators direct the air by creating a very gentle air movement through the plenum and ULPA Filter to achieve ISO Class 5 air quality in the chamber.

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 CO_2, humidity and temperature uniformity, homogeneity, and control.
- Circulation of filtered air passes across the water pan to accelerate the humidifying process.





b. Natural Convection (No Internal Fan)

The benefit is absolutely no vibration, but recovery time is typically much longer and the homogeneity and stability are not as accurate as the Esco CO_2 incubator.

D. Recovery Rates and Accuracy of Recovery to Program Set Point After Each Inner Glass Door Opening

Esco VivoCell™ Precise Parameter Control

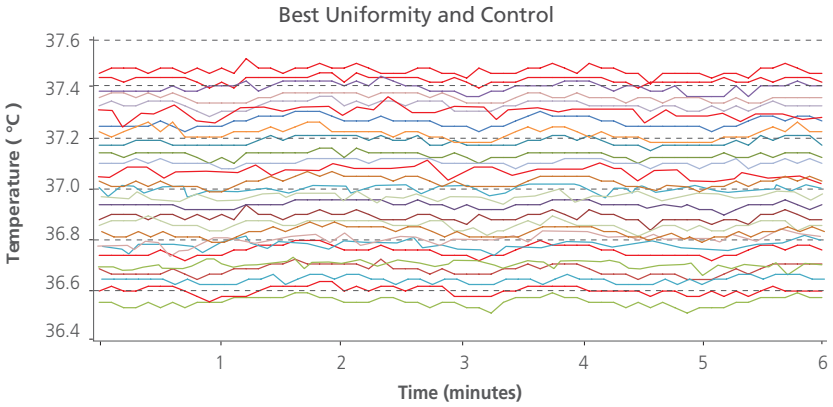


Figure 1. Different lines represent 27 sensors positioned inside the chamber. Esco CelCulture® CO_2 incubator has uniformity variance of less than ± 0.5 which means all the samples are evenly heated.*

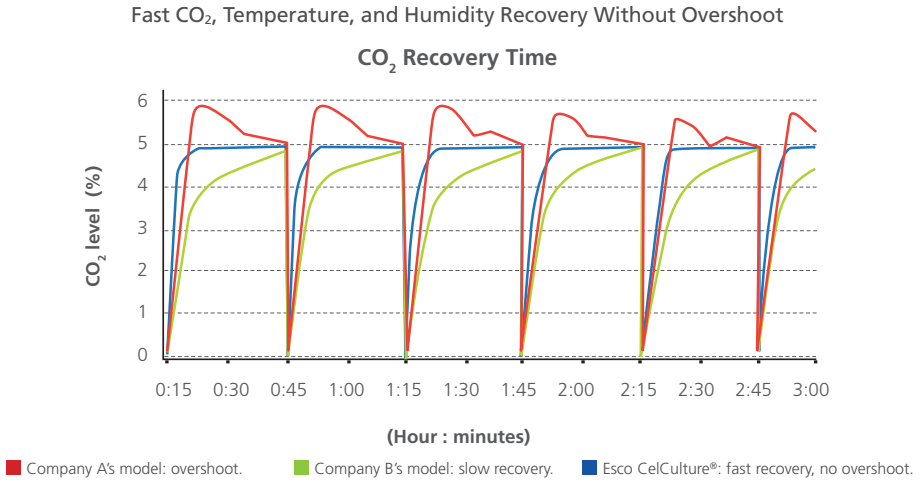


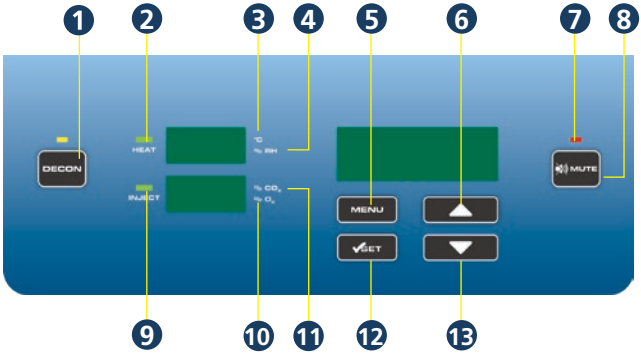
Figure 2. Precisely tuned sensor and software results in faster recovery of CO_2 without overshoot. This ensures uniform CO_2 levels even with frequent incubator door openings.

** Units were factory-tested under controlled environmental conditions per DIN 12880 standard and 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.*

E. Ease of Programming, User-Friendly Control System, and Built-In Data-Logging

In your selection process, it is important to carefully consider how user-friendly are the programming menus and other control parameters on the CO_2 incubator. Also, take into consideration if the CO_2 incubator has on-board diagnostic, help menus, and data-logging. Validation is now being used in the research laboratory environment. Considering this in advance before purchasing your CO_2 incubator may prove to be very prudent.

All Esco CO_2 incubator standard models have built-in Data-Logging, Diagnostic, and Help Menus. Units are simple to programme and navigate to other menus.



1. Start / stop decontamination cycle
2. HEAT LED lights when heat is applied to chamber
3. °C is lit when displaying the temperature
4. %RH is lit when displaying the humidity level
5. Enter menu / go back to previous menu
6. Scroll up / increase value
7. ALARMS LED will blink when errors and warnings occur
8. Mute alarms
9. INJECT LED lights when gas is injected
10. $\%O_2$ is lit when displaying the O_2 concentration
11. $\%CO_2$ is lit when displaying the CO_2 concentration
12. Confirm value / enter a menu
13. Scroll down / decrease value

F. User-Friendly Software Interface

Typically the common interface options are RS232, RS485, USB, SD Card, and Ethernet. These are some of the various options available on the market today. It is also important to consider how data retrieval and downloading works on a software suite package.

Esco offers a very user-friendly Voyager Software for continuous logging of incubator chamber's temperature, %CO_2, %RH, and %O_2 (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 Equipment

- CelCulture® - CO_2 Incubator (CCL)
- CelMate® - CO_2 Incubator (CLM)
- Lexicon® II - Ultra-low Temperature Freezer (UUS)
- Isotherm® - Laboratory Oven (OFA)
- Isotherm® - Forced Convection Incubator (IFA)
- Isotherm® - Refrigerated Incubator (IFC)
- Isotherm® - Natural Convection Incubator (INA)

G. Quality and Cleanliness of Atmosphere Inside Your CO_2 Incubator

It is very important to consider how to prevent the risk of contamination and cross-contamination between samples when culturing inside your CO_2 incubator. Many global CO_2 incubator manufacturers offer no means to ensure a clean air quality inside the inner chamber that may result in possible contamination of cell samples. Manufacturers of CO_2 incubator with no internal fan cannot possibly fit the filter, thus, air cleanliness is not guaranteed.

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 filter to provide enough protection as demanded by today's cell researchers and scientists. Therefore, Esco has provided a considerable step by becoming a global leader in manufacturing CO_2 incubator by fitting a standard ULPA filter.

Esco offers a built-in Ultra Low Particulate Air (ULPA) Filter to help minimize any airborne contaminants inside the incubator's chamber. This filter operates at 99.999% efficiency, superior to conventional HEPA filters which are 99.99% efficient, achieving ISO Class 5 cleanliness.

H. Types of Decontamination/Sterilization Methods

It is essential for research laboratories to culture their samples in an equipment without the risk of possible cross-contamination between cell cultures placed in a petri dishes, multi-well plates, flasks, and bottles.

Here are some of the commonly used contamination control methods:

a. 90 °C Moist Heat Decontamination Cycle

Has been proven effective in deactivating normally resistant fungi, bacterial spores, and vegetative cells. Esco offers this decontamination solution to different models of CO_2 incubators.

b. 180 °C High Heat Sterilization Cycle

Proven to be effective in killing normally resistant fungi, bacterial spore, and vegetative cells. Nontoxic and noncorrosive sterilization that completes within 12 hours leaving the chamber cool and dry at the end of the cycle. Esco offers this sterilization solution to CCL-HHS models.

c. Ultraviolet Light (UV) Lamp

Only effective and successful if all surfaces including shelves are fully exposed to the UV. UV lamp is isolated from the inner chamber via plenum cover, only exposing certain areas to be decontaminated. Growth of other contaminants to other parts of the chamber is possible if not fully exposed to the UV light. Esco offers a CO_2 incubator with UV lamp as an optional model.

d. Hydrogen Peroxide (H_2O_2)

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 neutralized back to water by the UV during the final stage of the cycle. Carefully conduct a risk assessment based on the number of units, size of your laboratory, and ventilation systems before purchasing CO_2 incubators with a hydrogen peroxide decontamination cycle.

e. No Sterilization or Decontamination Cycle

If no cycle is fitted (usually available on cheaper entry-level CO_2 incubators), then it is mandatory to routinely clean the inner chamber, shelves, shelf locators, plenum, ducting, fan blade, etc. by using a sterile cloth, 70% isopropyl alcohol, and distilled water solution. These types of CO_2 incubators can have a significant loss of samples over time. Therefore, it is highly recommended to consider buying an incubator with a decontamination or sterilization cycle.

A regular cleaning protocol should also be considered by the laboratory head.

The previous list describes many different types of sterilization and decontamination processes offered by various manufacturers of CO_2 incubators.

Esco offers the following automated decontamination/sterilization methods:

VALIDATED SWIFTCON™ 90 ˚C MOIST HEAT DECONTAMINATION CYCLE

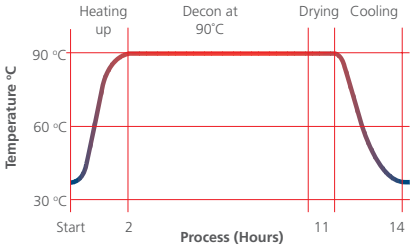


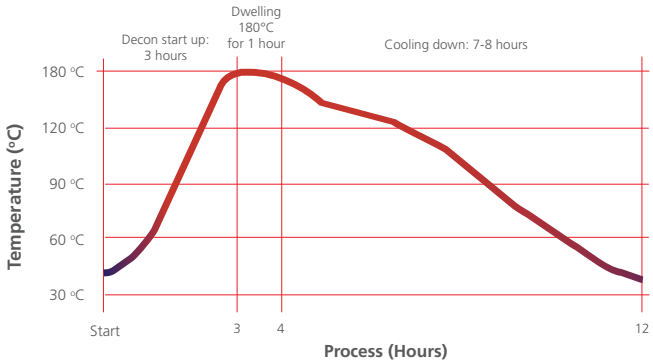
Figure 3. Graph of a full cycle decontamination process. Complete cycle lasts up to 15 hours.

Table 1. Log reduction of tested microorganisms after decontamination process.

Microorganisms	Before Decon	After Decon
Bacillus atrophaeus	1.59 x 10^6	0
Aspergillus brasiliensis	1.52 x 10^4	0
Pseudomonas aeruginosa	2.38 x 10^6	0
Staphylococcus epidermis	2.33 x 10^6	0
Escherichia coli	1.57 x 10^6	0
Staphylococcus aureus	5.72 x 10^6	0
Enterobacter faecalis	2.15 x 10^6	0

180°C HIGH HEAT STERILIZATION

Conforms to the International Standards for dry heat sterilization and has proven to be effective in killing normally-resistant fungi, bacterial spore, and vegetative cells. Nontoxic and noncorrosive sterilization that completes within 12 hours leaving the chamber cool and dry at the end of the cycle.



Results are achieved when tested at 37°C as set point in temperature ambient of 22 to 25°C. Results may vary if set point changes and calibration is needed.

Figure 4. Graph of a full cycle sterilization process. Complete cycle lasts up to 12 hours.

I. Built, Performance, and Quality Construction of Your CO_2 Incubator

a. Built

All Esco manufactured CO_2 incubators are subjected to the most stringent quality, testing, and calibration protocol. Esco believes it is extremely important to commit to intensive testing of every single CO_2 incubator. All new Esco CO_2 incubators are provided with all the necessary installation tools and gas tubing, securing clips, a detailed user manual, and the full factory test report data, including all checks undertaken and offsets entered. Esco ensures that each CO_2 incubator manufactured is of the highest standards and build quality.

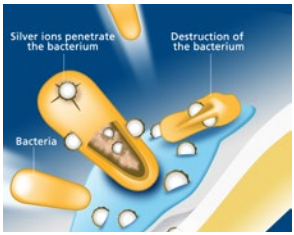
b. Performance

Esco is very confident that the Esco's wide range of CelCulture® CO_2 incubators is one of the most accurate in terms of homogeneity, uniformity, and control available on today's market.

c. Quality

It is important to consider the quality of the exterior finish of your CO_2 incubator. The presence of the external anti-microbial coating eliminates surface bacteria that may try to survive and grow on the external surfaces of your CO_2 incubator.

Esco CO_2 incubator exterior is made of electro-galvanized steel with white oven-baked epoxy-polyester antimicrobial powder-coated finish. External surfaces are powder coated with Esco Isocide™ to eliminate 99.9% of surface bacteria within 24 hours of exposure, ensuring a healthier, safer, and cleaner lab environment.



Isocide™ Antimicrobial Surface Coating

J. Wide Range of Options

It is important to consider the options you may need for your CO_2 incubator. Esco has a very comprehensive range of options.

a. Oxygen Control Option

Esco offers suppressed O_2 (Oxygen) option on most of the CO_2 incubator models from 50 L, 170 L, and 240 L. In certain circumstances and in typical applications such as cultivation of primary cell lines and in addition to the typical 5.0% CO_2 requirement, depleted O_2 levels are also required inside the chamber of the incubator:

- Arterial Blood 10% O_2
- Tumor 5% O_2
- Liver 5%-7% O_2
- Eye 10% O_2
- Lower Lung Tissue 15% O_2
- Stem Cells 5% O_2
- *In Vitro* Fertilization 5% O_2

Esco O_2 Sensor:

Esco's O_2 sensor is highly accurate with resistance to high temperature, utilizes long life, non-depleting sensor technology, and has an integral heating element to prevent condensation.

Zirconia O_2 Sensor

- Outstanding sensor accuracy
- Faster response time
- Long-term stability
- Moist heat-proof design

b. Multi-inner Glass Door Options:

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

SEALED INNER DOOR KIT WITH 4 GLASS DOORS

CCL-170_-_-IVF

c. Copper Inner Chambers:

Some researchers prefer to use optional 100% pure copper for inner chambers. Copper has been known for millennia to have antimicrobial properties. Esco offers an optional 100% pure copper interiors to provide additional protection for your precious samples.

CELCULTURE® CO_2 INCUBATOR WITH COPPER INTERIOR CHAMBER

CCL-240_-_-Cu

K. Testing & Certifications

For IVF applications, Esco CelCulture® CO_2 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® CO_2 incubator is listed by Underwriters Laboratory (UL) to meet the requirements of both the U.S. and Canada standards for electrical/mechanical integrity.

HPA Validated Decontamination Cycle

The Esco CelCulture® CO_2 incubator's 90 °C decontamination cycle has been evaluated and shown to be an effective method for deactivation of the normally resistant fungi and bacterial spores of *Aspergillus brasiliensis* and *Bacillus atrophaeus*, and the vegetative cells of *Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Enterobacter faecalis,* and *Escherichia coli*.

FDA-listed, Class II, 510k exempt medical device

Esco CelCulture® CO_2 incubator is listed as a medical device on the U.S. Food and Drug Administration (USFDA) Establishment Registration & Device Listing. It is classified as a Class II medical device under 21 CFR 884.6120 for assisted reproduction accessories and is exempted for the premarket notification 510(k) to market.

L. After-sales Service Support

Consider looking for a CO_2 incubator manufacturer that provides after-sales service support. Esco ensures that its service is on top of the line, providing standard services from calibration, and preventive maintenance of your CO_2 incubator to meet your individual needs, and to maintain its excellent working condition.

Click here for more service information or scan the code.

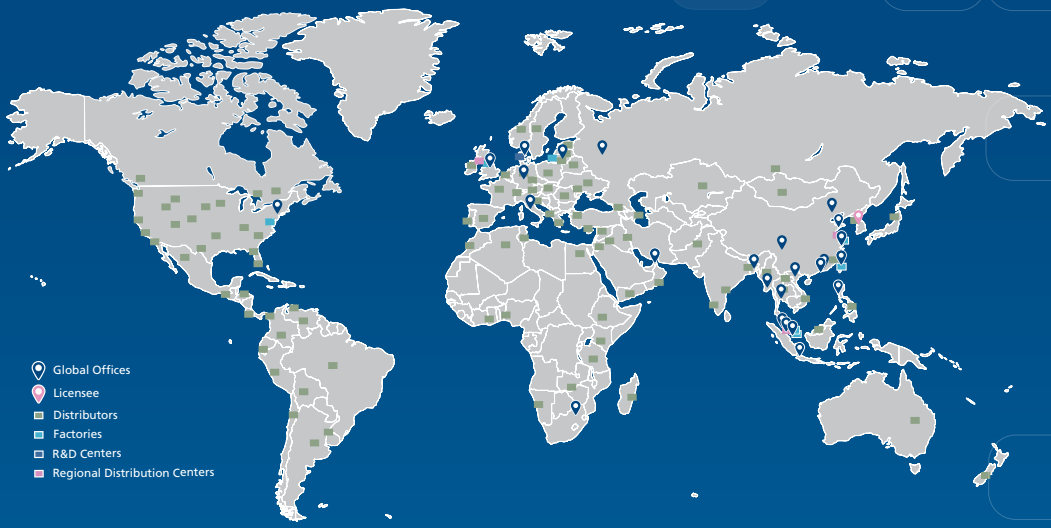
SCAN HERE

III. SUMMARY

Esco Lifesciences has undertaken lots of market research, analysed many competitors of CO_2 incubators, listened to our customers, and made every effort to produce a wide range of CO_2 incubator on today's market. We wish you every success in your CO_2 incubator selection and trust you will choose the right CO_2 incubator models in your selection process.

ESCO LIFESCIENCES GROUP

42 LOCATIONS IN 21 COUNTRIES ALL OVER THE WORLD



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