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CO_2 Resistant Orbital Shaker Selection and Comparison with Integrated Devices

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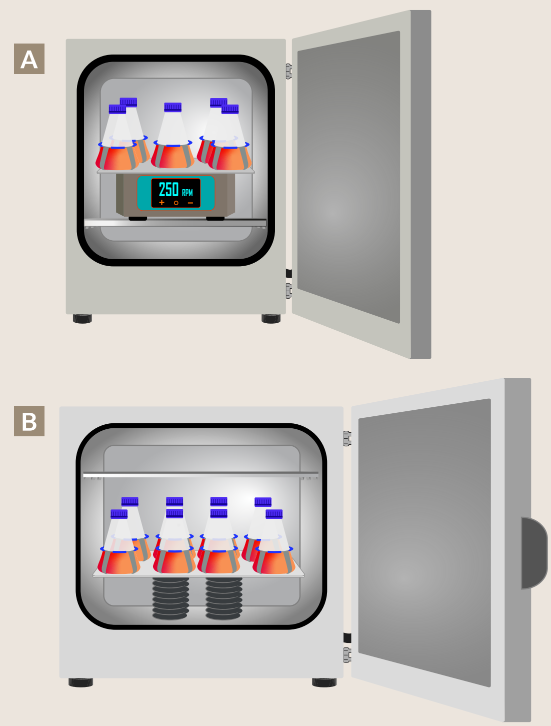
Executive summary

Mammalian cells in suspension are typically cultivated in shake flasks in a CO_2 enriched atmosphere. Due to the many advantages of mammalian shake flask culture, like ease of handling, low costs, and the possibility to grow to higher densities, it is widely used in the biopharmaceutical industry. Two systems are commonly used: a) orbital shakers that are placed inside a traditional CO_2 incubator and b) CO_2 incubators with integrated shaker. As there is a significant overlap of application for both systems, we will discuss advantages and disadvantages in this paper considering the following aspects:

> Growth conditions
> Cleanability and contamination prevention
> Capacity
> Longevity and reliability
> Device flexibility for various applications
> Initial investment and running costs



Two systems are commonly used for agitation of shake flasks in a CO_2-enriched environment
A: Orbital shakers that are placed inside a traditional CO_2 incubator
B: CO_2 incubator shakers

Introduction

In contrast to static adherent cell cultivation, agitation of shake flasks in a CO_2-enriched environment is used to culture mammalian cells in suspension. Cells are either free-floating – as single cells or aggregates – or attached to microcarriers. Applications include the cultivation of embryoid bodies, organoids, the preparation of a starter culture (inoculum) for subsequent larger cell production in bioreactors (scale up), or the expression of complex recombinant proteins. One of the most prominent examples of use is the production of therapeutic recombinant proteins, like monoclonal antibodies or protein-based vaccines, for example in HEK-293 or CHO cells that have been adapted to suspension cell culture.

To provide a CO_2-enriched environment for the shake flasks, two systems are commonly used: a) orbital shakers that are placed inside a traditional CO_2 incubator and b) CO_2 incubator shakers. In case of orbital shakers, the use of specialized CO_2 resistant orbital shakers is recommended. They have been developed to reliably perform in a highly humid (~95 % relative humidity) and slightly acidic atmosphere. Often, cleanability of these specialized devices is also improved compared to standard orbital shakers to meet the higher demand for contamination prevention in mammalian cell cultivation. In the following chapters, we will compare the two systems for the cultivation of mammalian cells in shake flasks regarding severals aspects.

Growth conditions

Reliable growth conditions are crucial to achieve optimal and reproducible cultivation results. In addition to proper CO_2 control to balance pH in the medium, precise temperature and relative humidity control are key when selecting a system to cultivate mammalian cells in shake flasks. The avoidance of vibrations is another factor to consider, especially when planning simultaneous cultivation of adherent cells in the same device. Here, we will directly compare both systems for agitated mammalian cell cultivation regarding the growth conditions.

CO_2 resistant orbital shaker inside a CO_2 incubator
For this system, the growth conditions are naturally determined by the performance of the surrounding CO_2 incubator. Thus, users should look for tight CO_2 (= medium pH) and temperature control specifications including stability, accuracy, homogeneity, and recovery after door opening (ideally < 5 min to 98 % of initial value after 30 s opening of an unsegmented inner door). The use of a device in an incubator chamber introduces additional heat to the chamber environment. As CO_2 incubators are thermally insulated and usually lack active cooling, a specialized CO_2 resistant orbital shaker with minimal heat dissipation is mandatory to reduce local temperature variances to a minimum. Thereby, the risk of condensation and temperature control failure by excess heat is minimized. One issue with the use of CO_2 resistant orbital shakers is related to their weight, which is usually in the range of 7 – 18 kg (15.4 – 39.7 lbs). Including maximum shaker loads of 2 – 6 kg (4.4 – 13.2 lbs), this weight can easily exceed the maximum shelf load of a CO_2 incubator and thus may lead to shelf bending and subsequent uneven cell growth when this shelve is also used for adherent cell cultivation.

Integrated device (CO_2 incubator shaker)
CO_2 and temperature control specifications for a CO_2 incubator shaker should be as close as possible to a typical CO_2 incubator for optimal performance. In addition, special attention should be given to the specified relative humidity of the device. Generally, the higher the relative humidity, the lower the medium evaporation and the lower harmful impacts by significant shifts in concentration of metabolites, salts, etc. Depending on device construction and humidification method, CO_2 incubator shakers can also achieve up to 95 % of relative humidity, like standard CO_2 incubators. In well-controlled systems and suitable ambient conditions, the risk for condensation is also low.

Compared to the use of an open-air shaker in an incubator, which transfers additional heat to the chamber, with an integrated device the risk of interfering with temperature control is excluded. Another advantage of an integrated device is the greater protection of simultaneously cultivated adherent cells from vibrations (details below under "Device flexibility for various applications"). The shaker drive is typically located outside the chamber and thus minimize the transfer of vibrations to the shelves.

Summary - Recommendations for CO_2 resistant orbital shaker selection:

- Look for tight CO_2 and temperature control specifications of the CO_2 incubator including stability, accuracy, homogeneity, and recovery after door opening (ideally < 5 min to 98 % of initial value after 30 s of unsegmented door opening).
- The CO_2 resistant orbital shaker should come with minimal heat dissipation.
- Keep in mind that shelves may bend under the shaker load. This may turn the shelves unusable for reproducible adherent cell culture due to varying medium levels above the culture.
- Ideally, test the performance of your CO_2 incubator with the CO_2 resistant orbital shaker of choice to ensure it doesn´t interfere with temperature control and airflow.
- Is parallel static cell cultivation inside the same device planned? Especially if vibration-sensitive cells like stem or primary cells are concerned, consider a dedicated device.

Cleanability and Contamination Prevention

The warm, humid atmosphere and nutrient-rich media used for mammalian cell cultivation provide ideal growth conditions for harmful and sometimes hard to detect contaminants like Mycoplasma spp. An additional challenge are cross-contaminations by eukaryotic cell lines. These can be introduced into cultures by earlier or simultaneous projects inside the same device.

Regardless of its type, the total costs of contamination may exceed the device's purchase price many times; additional expenses are incurred through the loss of sample material, the need to repeat experiments, combatting the contamination itself, and possible downtimes imposed by these measures. Therefore, a contamination must be avoided at all costs by constantly applying aseptic techniques in the lab. In addition, because of the significant impact of a contamination and because of the labor invested in cleaning over the lifetime of a device, it is worth it to think about a device-intrinsic structural defense. Here, we will review the most important structural factors to consider for both systems regarding cleanability and contamination prevention.

Number and structure of internal parts

Contaminants preferably grow on moist surfaces or in liquids. Therefore, the chamber interior should facilitate the detection and fast, thorough removal of medium spills. In addition, to ensure the recommended weekly removal of moist dust and dirt on internal parts, the psychological threshold for cleaning should be low ("What is easy to do is more likely to get done."). Put simply, the more parts and more crevices inside a device, the harder and less likely it is to detect/remove a moist contamination nucleus.

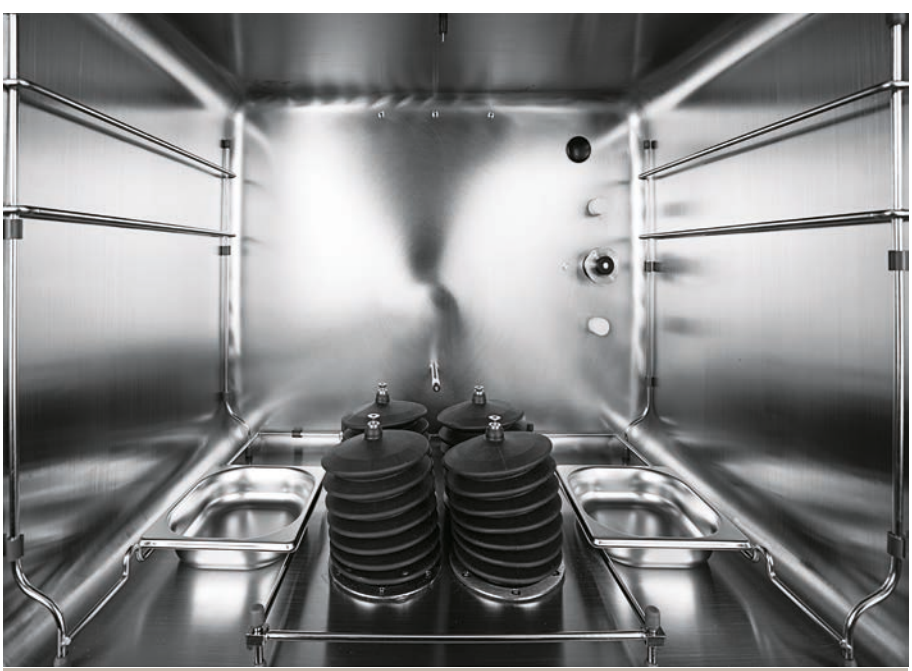
To ensure the exclusion of moisture inside the device, sealed open air CO_2 resistant orbital shakers are favorable over standard open air orbital shakers. However, regardless of its type, a shaker inside a CO_2 incubator adds significantly to the internal complexity and vulnerable surface area, thus reducing the cleanability.

Humidification and chamber design

The high humidity inside a device is often provided by a water tray. It is easy to refill and clean, but it is also a risk factor for contamination. Additives are not a suitable solution due to their corrosive properties. Therefore, the water tray should be removable to ensure weekly water removal, drying and cleaning with disinfecting agents.

The weld seams of the chamber wall can be a welcoming place for contaminants to hide in. Therefore, seamless (deep-drawn) chambers should be preferred. Seamless designs also reduce the time for proper cleaning.

For the surrounding CO_2 incubator or integrated devices, seamless (deep-drawn) chambers without a fan are recommended. This reduces the number of internal parts, thus hidden places for contaminants, and make cleaning easier.

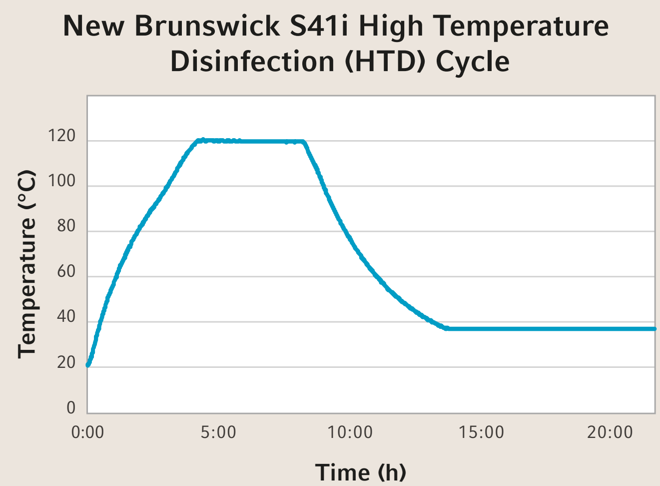


The seamless, deep-drawn chamber of the New Brunswick S41i with removable water trays. The high temperature-resistant rubber bellows protect the connection of the shaker drive with the platform (not installed here).

High temperature disinfection routine

For frequent disinfection, a dry heat routine for several hours has been established as the gold standard in CO_2 incubators. For integrated devices this routine is available, but still an exception.

Ideally, to disinfect the CO_2 resistant orbital shaker and all internal parts, they should be resistant to the temperatures used (120 – 180 °C). However, there are no open air orbital shakers available on the market that are resistant to these temperatures. Additionally, depending on the CO_2 incubator used, heat-sensitive sensors or fan-associated HEPA-filters may need to be removed before the routine. Not only are the orbital shaker and these parts not disinfected by the routine, they also pose a high risk of (re)introducing contamination due to a prolonged reinstallation with an open door after the device sterilisation routine. Thus, if a high degree of contamination prevention is key, an integrated device with high temperature disinfection is recommended as it can include all internal parts in the routine (possible exceptions: adhesive mats or non-metal girdles on shaker clamps).



Standard in modern CO_2 incubators, exception for integrated devices: high temperature disinfection cycle.

Summary - Recommendations for CO_2 resistant orbital shaker selection:

> Electronics and drive-related mechanical parts should be sealed and thus protected from spills.
> The surrounding CO_2 incubator should come with:
  > a low number of internal parts that need to be removed,
  > a simple, non-labyrinthine structure of these internal parts,
  > a low number of chamber crevices, and
  > an easy to remove and clean water tray (avoid integrated water trays).
> Test the performance of the CO_2 incubator with the CO_2 resistant orbital shaker of choice to ensure it doesn´t lead to an increased contamination risk by condensation (and other issues – see other chapters).
> An integrated high temperature disinfection routine is recommended regardless of the setup and should include as many internal parts as possible.

Capacity

One of the key considerations when choosing between the two systems is the targeted throughput within the next years of the lab because both differ significantly in this regard. Generally, CO_2 resistant orbital shakers are limited to low throughput and small flask volumes up to 1 L due to two reasons. First, platforms are small with dimensions usually in the range of 36 x 30 cm (14.2 x 12.8 in) resulting in an area of 1,080 cm^2 (425 in^2). Integrated devices come with at least double and up to more than four-fold platform areas. Second, drives for CO_2 resistant orbital shakers have been developed for maximum loads of typically only 2.5 – 6 kg. Integrated devices can be used with a significantly higher load.

Longevity and Reliability

Protection of mechanical parts and electronics

In an integrated device, drive-related mechanical parts and electronics, except for sensors, are located outside the chamber. Therefore, in these devices, crucial parts are protected from the potentially corrosive humid and CO_2 enriched, slightly acidic growth atmosphere.

In contrast, mechanical parts and electronics of small orbital shakers are exposed to the challenging atmosphere. In addition, because devices inside a CO_2 incubator can significantly interfere with temperature control and airflow, condensation may occur. Thus, it is again advisable to select a specialized sealed CO_2 resistant orbital shaker and preferably test it for interference with the CO_2 incubator before buying. For added resistance of the shaker against the atmosphere, the exterior should be made of corrosion-resistant materials. Suitable materials include stainless steel or specialized plastics like Acrylonitrile butadiene styrene (ABS) that is also used, for example, to produce LEGO® bricks. In addition, electronics and drive parts of the orbital shaker should be sealed and protected from spills.

Humidity resistance of the control unit

A less known aspect that is specific to selecting a suitable CO_2 resistant orbital shaker, is related to the humidity resistance of the control unit. Some manufacturers limit the reliable operation to 60 % relative humidity, which may become problematic, especially in labs without air conditioning. Therefore, it is worth the effort to compare the manufacturer´s operating manual with the expected relative humidity before buying.

The shaker drive

The centerpiece of every shaker is it´s drive. Its reliable performance makes the difference between peace of mind and repeated weekend work. Therefore, especially when planning to agitate loads close to the device´s limits 24/7, special attention should be paid to the construction of the shaker drive. Additionally, it is advisable to consider choosing an integrated device with a robust drive built to reliably sustain high loads over its lifetime, especially when having a high throughput, working with larger volumes and higher rpms.

If unexpected downtime and delayed results are not an option for you, a look at the shaker´s heart is recommended. For example, heavy triple eccentric shaker drives (image) are made for stable, uniform, and vibration-free motion of high loads 24/7.

Summary - Recommendations for CO_2 resistant orbital shaker selection:

> Exterior should be made of corrosion-resistant materials like stainless steel or ABS.
> Electronics and drive-related mechanical parts should be sealed and thus protected from spills.
> Check the ambient conditions of the device´s control unit in the operating manual, if a relative humidity of the lab >60 % cannot be excluded (e.g. in labs without air conditioning).
> Ideally, test the performance of your CO_2 incubator with the CO_2 resistant orbital shaker of choice to ensure it doesn´t interfere with temperature control and airflow.
> If the targeted load is close to the device´s limit consider choosing an integrated device.

Device flexibility for various applications

Both systems offer a certain flexibility for other applications outside the primary use discussed so far. With a CO_2 resistant orbital shaker, it is possible to use the shaker and the CO_2 incubator as standalone devices for non-incubated shaking and adherent cell culture, respectively. If the shaker is also intended to be used for protocols that include high speed agitation (>200 rpm) of plates and tubes, it is advisable to check the operating manual beforehand – some devices have a shaking speed limit of 200 rpm.

Parallel static cell cultivation

One major disadvantage of CO_2 resistant orbital shakers is their limited use for parallel static cell cultivation inside the same device. In contrast to integrated devices, the shaker drive is located inside the chamber and likely transfers vibrations to the shelves above. Vibrations interfere with the cell´s adherence, which may lead to unnatural growth patterns, a change in the cell´s metabolism and unreproducible results, especially for vibration-sensitive cells like primary or stem cells[1]. A corresponding note regarding this limitation can also be found in the operating manual of some devices. If a parallel static cultivation is desired, the setup should be tested before buying. This test should include proper positioning on a suited shelf and check for vibrations in the chamber when reaching the targeted rpm. Alternatively, an integrated device can be considered as these have been developed (well-balanced shaker drive outside the chamber) and usually tested for shaking and parallel static cell cultivation. Almost all integrated devices in the market come with the option of least one static shelf.

Summary - Recommendations for CO_2 resistant orbital shaker selection:

> Are protocols of rpm > 200 with plates and tubes planned? If yes, check the maximum rpm in the operating manual of the shaker before buying.

> Is parallel static cell cultivation inside the same device planned? Especially if vibration-sensitive cells like stem or primary cells are concerned, consider a dedicated device.

Initial invest and running costs

The ever-present economic pressure to attain greater productivity with finite resources and rising costs is also a reality in modern cell culture labs. Amongst many other factors, running costs for lab equipment are a significant factor when minimizing overall lab expenses. Therefore, purchasing decisions must consider not only initial device acquisition costs, but also the total cost of ownership over the expected lifetime of equipment.

Capacity matters

In absolute terms, the initial investment for a CO_2 incubator plus CO_2 resistant orbital shaker is generally lower compared to an integrated device. This is certainly even more true, if a CO_2 incubator already exists in the lab, as the price for a CO_2 resistant orbital shaker is usually in the range of $2,400 – 3,300. If low volumes of cells or low amounts of the cell culture product are necessary, and no capacity expansion is planned in the future, a CO_2 resistant orbital shaker is a cost-efficient solution. However, the cost comparison quickly changes in favor of an integrated device when costs per cell number or amount of cell product are compared.

Often underrated, CO_2 consumption can be a significant cost factor over the lifetime of a device. This can be due to the costs of gas itself, but also due to the necessary employment of staff to frequently change gas cylinders. In addition to the cost factor, corporate carbon footprint reduction goals sometimes also need to be considered in this regard.

1 Kanie K. et al, Effect of mechanical vibration stress in cell culture on human induced pluripotent stem cells, *Regenerative Therapy*; Vol. 12: 27–35. 15. Dec. 2019

Summary - Recommendations for CO_2 resistant orbital shaker selection:

> What capacity do you need in the next years? If you might exceed the maximum capacity also consider an integrated device.
> Are specialized reinforced incubator shelves necessary to hold the weight of the shaker plus load?
> Relevant for some CO_2 incubators in the market: Do access ports exist to pass the shaker´s power cord through the wall of the device? If not, significant costs may arise from service labor to create these access ports.
> If the power cord or the cable to the control unit is passed through the door sealings, an increased gas consumption is likely. This leads to increased costs for gas and increased labor for changing gas cylinders.
> Generally, it is advisable to compare the gas consumption of different CO_2 incubators and integrated devices of interest before buying.
> Consider the labor costs for proper frequent cleaning. How much time will the lab staff need to spend on this over the lifetime of the device? A seamless chamber, few internal parts and an integrated high temperature disinfection routine will save time for reliable cleaning and lower the risk of sample loss and contamination-related downtime.

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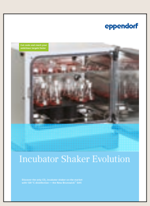

Brochure: Incubator Shaker Evolution: Discover the New Brunswick S41i CO_2 Incubator Shaker

White paper: CO_2 Incubators – Making the Best Choice for Your Lab

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