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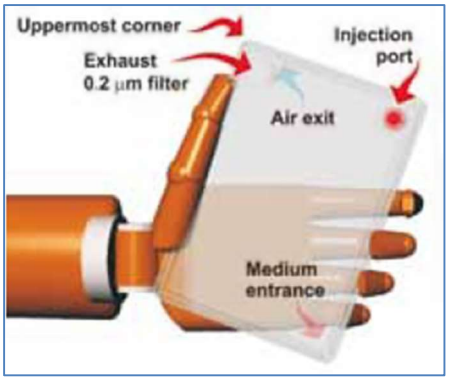
Petaka G3™ Applications: Frequently asked Questions

Why must I hold the Petaka G3™ upright when filling with medium?

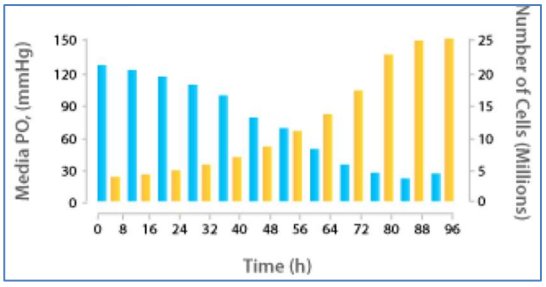
The restrictive, respiratory microchannel in the Petaka G3™provides physiological levels of O2 while simultaneously maintaining ideal CO2 and pH levels for your culture. However, this delicate balance of inward and outward gas diffusion is not optimal when the microchannel is filled with medium. To avoid this from happening, simply keep the Petaka G3™ upright when filling (**see Fig. 1**). The air path from the culture chamber to the gas microchannel is on the upper right corner of the Petaka, near the injection port. So, if you are careful to not let liquid into the capillary breakers or the microchannels your culture will soon enjoy ideal, in-vivo like gas control. No tri-gas incubators, glove boxes, or specialty environmental control chambers required!



The locations of main parts of the Petaka G3™, closed cell culture cassette.

At what level of dissolved oxygen will the Petaka G3™ maintain my cultures?

The proprietary microchannel gas regulator within each Petaka G3™will reduce the dissolved oxygen from hyperoxic levels (150mmHg pO2) to levels found in native tissues (25-50 mmHg pO2). The exact level of dissolved oxygen will vary depending on cell type, length of uninterrupted culture and medium conditions. It is important to understand that oxygen flow into the Petaka G3™ is controlled by diffusion, so the bigger the difference between the outside air and the inside culture chamber, the more oxygen will diffuse in. So, as the internal oxygen level is reduced, more oxygen will automatically and passively diffuse inward to maintain equilibrium.



As cells divide and grow in Petaka G3™ dissolved oxygen levels decrease to physioxic levels.

Just as in native tissues, cells in the Petaka G3™ will take what they need and establish their preferred local dissolved oxygen levels. The Petaka G3™ does not force an artificial level of dissolved oxygen on the cells in culture, this is why we state oxygen is “auto-regulated” and cells maintain physiologic levels of oxygen at all times.

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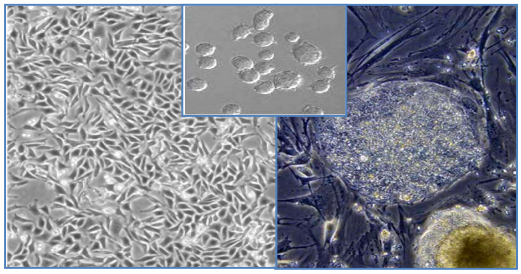
What types of cells can I culture in the Petaka G3™?

Practically every cell type has been successfully cultivated in the Petaka G3™, including attached, suspension, immortal, primary and transfected cells, hybridomas, MSCs, ESCs, iPSCs, etc. The Petaka G3™ FLAT is untreated for suspension cell culture while the Petaka G3™ LOT is surface treated for attached cell culture.



The Petaka G3™ works well with every cell type.

The Petaka G3™ is not just another flask; it corrects the error of cultivating cells in the harmful, hyperoxic conditions typical with plates, dishes and flasks. Since most cell lines have been cultivated in hyperoxic conditions for decades, Petaka users may observe differences as the cells re-adapt to their correct, physiological environment. Practically speaking, this can result in slight morphological changes, changes in doubling time, etc., but this is not a negative effect. It is actually corrective, and in the long-term your cells will be happier and more reflective of the native tissues when grown inside the Petaka G3™.

How is it possible to grow cells in the Petaka G3™ without supplemental CO2?

Cells naturally generate CO2. However, in plates, dishes and flasks this CO2 rapidly diffuses out of the medium, so a closed CO2 incubator system is required. The Petaka G3™ automatically retains CO2 at a level sufficient to maintain pH in a standard bicarbonate-buffered medium. However, a minimum number of cells in the starting culture must be present to supply sufficient CO2 to maintain this pH balance. Decades of experience has led Celartia to recommend at least two-million cells (25,000 cells/cm^2) be seeded in a Petaka G3™ when culturing without supplemental CO2. With this number of starting cells, your culture should have no problem maintaining excellent pH of the medium. Much lower cell densities will also do fine in the Petaka but may require supplemental CO2 for the first 24-hours of until sufficient cell numbers are reached and sufficient internal CO2 is generated.

How do I perform long-term microscopy with the Petaka G3™?

Because the Petaka G3™ provides a unique, closed, gas-controlled culture environment without significant evaporation, it acts like a mini-incubator all by itself. Because of this, specialty cell culture and experimental processes may be performed for long periods of time outside of the standard CO2 incubator environment. This is impossible with plates, dishes, flasks or bags since medium will evaporate leading to loss of osmolarity and the dissolved CO2 will rapidly be lost resulting in cell death.

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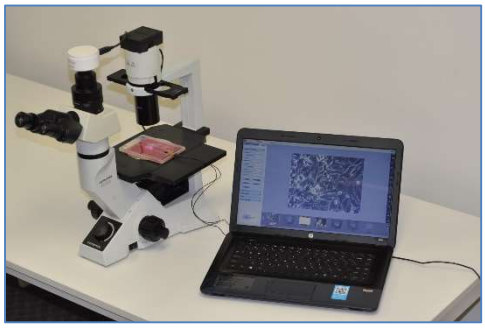
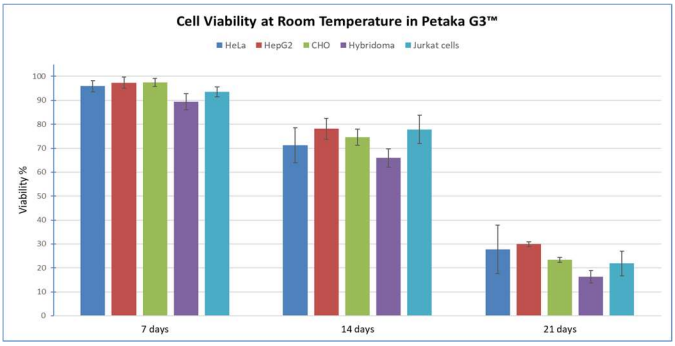
The Petaka G3™ supports functions like time-lapse microscopy without any special (and expensive) microscope incubator equipment. All one needs to provide is warming for the Petaka G3™, everything else is automatically maintained by the device (see our long-term benchtop microscopy setup, right). At Celartia, we routinely use small heating films to maintain cultures at 37°C on the microscope to record multi-day microscopy observations or to record pO2 in the medium on the benchtop. These films are available from Celartia, just ask!



Bench-top, time-lapse microscopy set-up with Petaka G3™ requires no incubator.

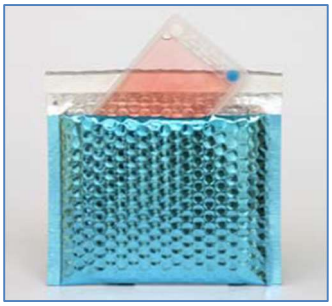
How do I preserve cells at ambient temperature in the Petaka G3™?

One of the amazing features of the Petaka G3™ is its ability to preserve cells at room temperature for extended periods of time. Many labs use the Petaka G3™to eliminate cryopreservation for processes that benefit from cell storage for up to 2-weeks. When cells are maintained at low dissolved oxygen, ideal pH levels, and ambient temperature (15-23°C), cells simply go dormant, pausing their cell division and reducing their metabolic rate significantly.



Viability of common cell lines during dormancy in Petaka G3™.

Most cells in this state are healthy and remain viable for 2-weeks or more. To wake these cells, simply return them to a 37°C incubator and allow them to warm…that's all. This is only possible because even outside of an incubator the Petaka G3™ maintains low oxygen and balanced CO2 levels. Combined with a sub-euthermic temperature and the cells are gently and perfectly maintained in a dormant state. Labs use this free process time to temporarily store cells or ship cells anywhere in the world.



Cells in Petaka G3™, in mailer.

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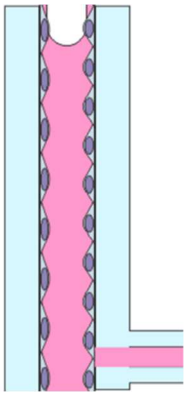
How do I cryopreserve cells in a monolayer in the Petaka G3™, and why is it superior to standard freezing in cryovials?

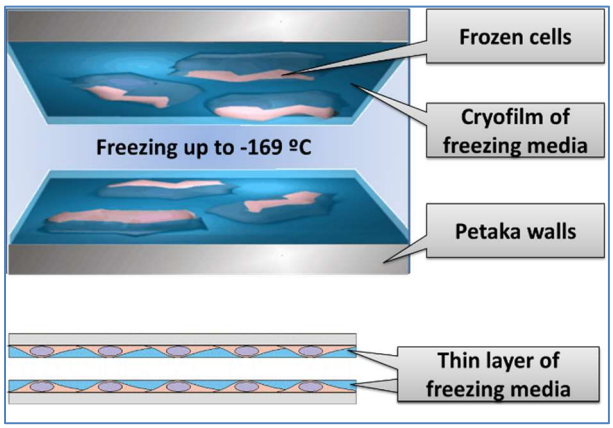
The Petaka G3™ is the only cell culture device that also allows for convenient and direct cryopreservation inside the primary culture device. Cryo vials feature a low surface-to-volume ratio. This means that the operator must use a slow freezing process while the glass transition progresses from the outside to the center of the vial. During this slow freezing period ice crystals may form inside of cells and damage them, leading to poor post-thaw viability. A preferred method is thin-film cryopreservation, only possible in the Petaka G3™. To freeze cells grown in a monolayer in the Petaka G3™ simply remove culture medium, rinse cells briefly in freezing medium, then aspirated out excess freezing medium. This leaves only a thin film of freezing medium over a layer of cells no more than 30um thick. This thin film may then be immediately flash frozen (vitrified) for permanent cryopreservation. This process prevents trypsin exposure, and all risks and damage associated with the traditional slow-freezing process.



Thin film production in Petaka G3™.

To thaw vials, they must be warmed in an unsanitary water bath for 1-2 minutes. This poses a major risk to sterility and this extremely slow thawing technique leads to even more cell death. In contrast, thawing cells in a Petaka G3™ is achieved simply by filling it with warm medium. This means each cell thaws in less than a second as the warm medium covers the frozen cell; no water baths, running water, or sample transfers required. After adding warm medium, cells in the Petaka G3™ go directly to culture, with no reattachment time or trypsin damage.

Moreover, up to 25 million attached cells may be frozen in a single Petaka G3™, using just 1ml of residual freezing medium. This means that when 24ml of warm medium is added to a Petaka G3™, the standard 10%DMSO is diluted to around 0.4%. This level of DMSO is no longer damaging to cells in culture, so cells thawed in a Petaka G3™ never need to be spun-down or washed during initial recovery and incubation. Cryopreservation in the Petaka G3™ means the whole process is faster, easier, carries less contamination risk, and leads to much higher post-thaw cell viability.



Attached cells in Petaka G3™, frozen in a thin film results in superior cell viability compared to cryofreezing with vials.

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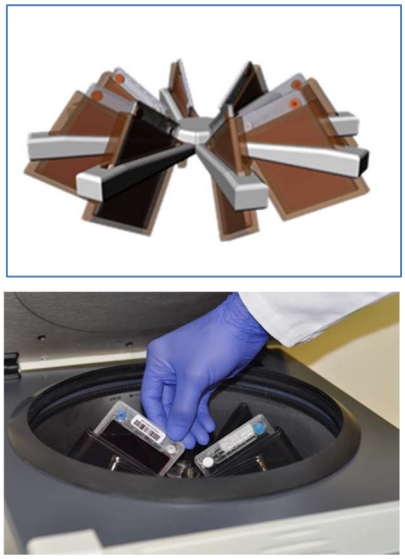
How is it possible to wash and centrifuge cells without transfer to conical tubes?

The Petaka G3™ is the only cell culture device that can be centrifuged directly. This allows the user to pellet, wash and separate cells without any transfers into conical tubes. Unlike open dishes, plates, and flasks, the Petaka G3™ is designed and manufactured to withstand up to 650g during routine centrifugation. So, instead of aspirating and transferring cells for centrifugation, the user can simply place the Petaka G3™ into the centrifuge and spin directly. This results in a cell pellet in the bottom corner of the Petaka G3™. These cells stay put as media is aspirated off and fresh media is added. Likewise, the pelleted cells may be selectively harvested while the old media is retained. This eliminates sample transfers into in and out of centrifuge tubes, and practically eliminates contamination risk. Old Traditional limitations and cell culture dogma no longer apply; everything is now possible with the Petaka G3™.



Centrifugation in the Petaka G3™rotor.

What do the colored ports on the Petaka G3™ indicate?

The Petaka G3™ LOT for adherent cells features a blue silicone injection port. The Petaka G3™ FLAT for suspension cells features a white silicone injection port. The orange color port on the Petaka G3™ HOT is for when you want slightly higher oxygen tension in your cultures.

Should I culture with the Petaka G3™ in an upright or horizontal configuration?

Suspension cells must be cultured in the Petaka G3™ in a horizontal configuration, similar to a flask because these cells will clump on the bottom when the Petaka G3™ is cultured upright. After adherent cells attach, (typically 1-2 hours), the Petaka G3™ may be left in the horizontal position or it may be switched to an upright position for the duration of the culture. However, when the Petaka G3™ is kept "horizontal" it is strongly advised to elevate the top end of the Petaka G3™ slightly higher than the bottom (5°-15°) to limit the amount of medium that may enter the capillary breakers at the top of the Petaka G3™.

The preferred horizontal configuration with the top end slightly higher than the bottom end.

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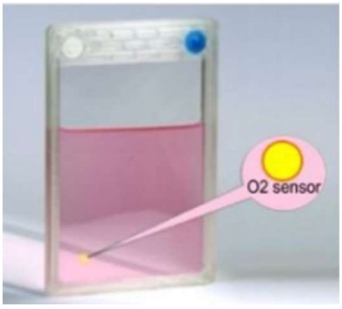
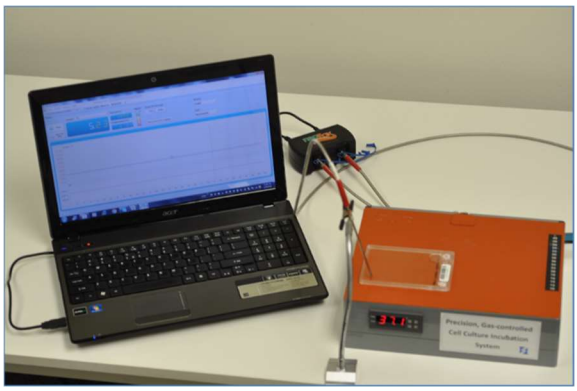
How can I measure the amount of dissolved oxygen inside my Petaka G3™?



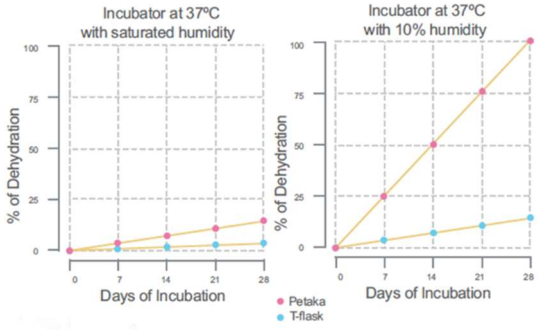
The Petaka G3™ is unique in that it is now possible to measure the dissolved oxygen (pO2) in your culture in real time, using a sterilize, off-the-shelf setup. The Petaka G3™ DO Sense features an oxygen sensing dot manufactured by Ocean Insights, that when paired with the NeoFox™ reader, allows the user to measure and record the dissolved oxygen in the culture medium with precision and high temporal resolution. With the Petaka G3™ DO Sense it is easy to perform simple bioprocess monitoring or to carry out sophisticated experiments determining single cell oxygen consumption rates.

The setup is simple. Simply fill the Petaka G3™ DO Sense with your cell culture and incubate on any 37°C warming surface. This is only possible in the Petaka G3™ because every other culture device requires a humidified CO2 incubator. After filling, position the NeoFox™ reader to align with the sensing dot and start measuring. That's all there is to it. The user will then observe the classic pO2 reduction curve as the cells consume excess dissolved oxygen. The user can observe and record the culture progressing in real time from an artificial, hyperoxic state to a more natural and relevant, physioxic condition. Labs can maintain this culture in open air for days since there is practically no media loss due to evaporation and the pH of the culture is perfectly maintained without supplemental CO2.



How is it possible to eliminate the water tray from my incubator?

Standard plates, dishes and flasks must be open to allow open CO2 gas exchange. The Petaka G3™ is functionally closed and retains CO2 in culture automatically. All liquid transfers are performed through a closed, sterile silicone port. In addition to benefit of reduced contamination risk during processing, the closed design means that media evaporation is practically eliminated. This is why there is no need to maintain a water tray inside the incubator. This eliminates a second major contamination source.



The Petaka G3™ has been shown to minimize evaporation loss in culture even at extremely low ambient humidity levels.

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What are the basic features of the Petaka G3™?

- Class VI medical grade resin
- Optically clear polystyrene growth surface
- Sterile (SAL 10-6)
- Standard SBS footprint
- 0.2µm PTFE air filter
- 24ml filling volume
- 95kPa tested
- Free of detectable endotoxin
- Sterile silicone port access
- 75cm2 surface (one-sided culture)
- 150cm2 surface (double-sided culture)
- Functionally closed processing
- Practically no media loss due to evaporation
- Single-use disposable