Frequently Asked Questions (FAQs): Petaka G3™ Cell Culture System

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

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

The Petaka ™ is not just another flask; it corrects the error of cultivating cells in the non-physiological, 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 cells when grown inside the Petaka ™.

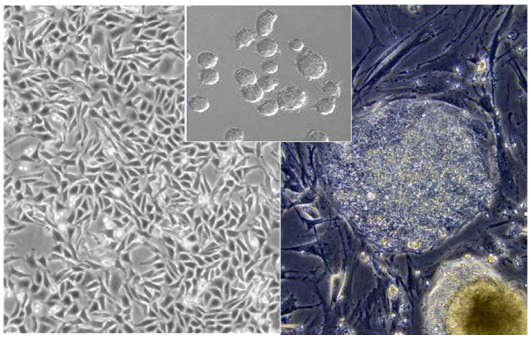

Figure 3. The Petaka ™ works well with every cell

Can I culture hybridoma cells in the Petaka ™?

Yes, the Petaka ™ HOT has been developed specifically for cells requiring high levels of oxygen, including hybridomas and other cell types that thrive in artificially high oxygen conditions.

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

One of the amazing features of the Petaka ™ is its ability to preserve cells at room temperature for an extended period of time. Many labs use the Petaka ™ 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.

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. This is possible because even outside of an incubator, the Petaka ™ maintains low oxygen and balanced CO_2 levels which

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combined with a sub-euthermic temperature maintains the cells in a dormant state. Labs use this process to store or ship cells anywhere in the world.

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

Suspension cells must be cultured in the Petaka ™ in a horizontal configuration similar to a flask, because these cells will clump on the bottom when the Petaka ™ is cultured upright. After adherent cells attach, (typically 1-2 hours), the Petaka ™ may be placed in the horizontal position or it may be switched to an upright position for the duration of the culture. However, when the Petaka ™ is kept "horizontal" it is strongly advised to elevate the top end of the Petaka ™ 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 ™.

Figure 6. The preferred horizontal configuration.

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

The restrictive, respiratory microchannel in the Petaka ™ provides physiological levels of O_2 while simultaneously maintaining ideal CO_2 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 ™ upright when filling. The air path from the culture chamber to the gas microchannel is on the upper right corner of the Petaka G3™, near the injection port. By ensuring that liquid is not permitted to enter the capillary breakers or the microchannels, your culture will reach ideal, *in vivo* like gas control. No need for tri-gas incubators, glove boxes, or speciality environmental control chambers.

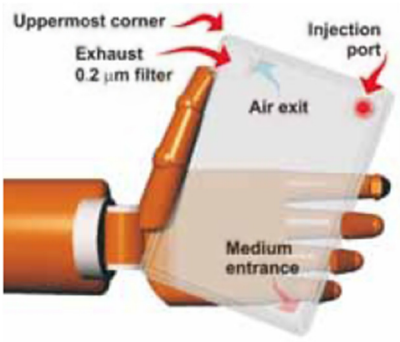

Figure 1. Shows the holding position of the Petaka ™ during operations.

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

The proprietary microchannel gas regulator within each Petaka ™ will reduce the dissolved oxygen from hyperoxic levels (150 mmHg pO_2) to levels found in native tissues (25-50 mmHg pO_2). The exact level of dissolved oxygen will vary depending on cell type, length of uninterrupted culture

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and medium conditions. It is important to understand that oxygen flow into the Petaka ™ is controlled by diffusion, so the bigger the difference between the outside air and the inside culture chamber, the more oxygen will diffuse in. Therefore, as the internal oxygen level is reduced, more oxygen will automatically and passively diffuse inward to maintain equilibrium.

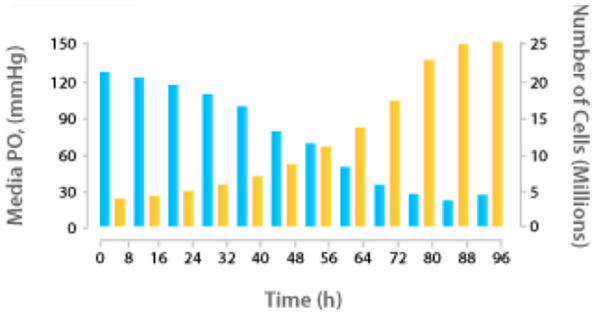

Figure 2. Shows as cells divide and grow in the Petaka ™, dissolved oxygen levels decrease to physioxic levels.

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

How is it possible to grow cells in the Petaka ™ without supplemental CO_2?

Cells naturally generate CO_2. However, in plates, dishes and flasks this CO_2 rapidly diffuses out of the medium, so a closed CO_2 incubator system is required. The Petaka ™ automatically retains CO_2 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 CO_2 to maintain this pH balance. We recommend at least two-million cells (approximately 25,000 cells/cm^2) be seeded in a Petaka ™ when culturing without supplemental CO_2. With this number of starting cells, your culture should have no problem maintaining the pH of the medium. Much lower cell densities can also be cultured in the Petaka ™ but may require supplemental CO_2 for the first 24 hours, or until sufficient cell numbers are reached and sufficient internal CO_2 is generated.

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

Because the Petaka ™ provides a unique, closed, gas-controlled culture environment without significant evaporation, it acts like a mini-incubator by itself. Because of this, speciality cell culture and experimental processes may be performed for long periods of time outside of the standard CO_2 incubator environment. This is impossible with plates, dishes, flasks or bags since medium will evaporate leading to loss of osmolarity and the dissolved CO_2 will rapidly be lost resulting in cell death.

Figure 4. Bench-top, time-lapse microscopy set-up with Petaka ™ requires no incubator.

The Petaka ™ supports functions like time-lapse microscopy without any special (and expensive) microscope incubator equipment. All you need to provide is warming for the Petaka ™, everything else is

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automatically maintained by the device. We routinely use small heating films to maintain cultures at 37°C on the microscope to record multi-day microscopy observations or to record pO_2 in the medium on the benchtop.

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

The Petaka ™ 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 centre 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, which is only possible in the Petaka ™. To freeze cells grown in a monolayer in the Petaka ™, simply remove culture medium, rinse cells briefly in freezing medium, then aspirate out excess freezing medium. This leaves only a thin film of freezing medium over a layer of cells no more than 30 µm 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.

To thaw traditionally frozen cells in cryo-vials, they are typically warmed in a water bath for 1-2 minutes, which poses a risk to sterility. Thawing cells in a Petaka ™ is achieved 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 ™go directly to culture, with no reattachment time.

Moreover, up to 25 million attached cells may be frozen in a single Petaka ™, using just 1 mL of residual freezing medium. This means that when 24 mL of warm medium is added to a Petaka ™, 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 ™ never need to be spun-down or washed during initial recovery and incubation. Cryopreservation in the Petaka ™ means the whole process is faster, easier, carries less contamination risk, and leads to a high post-thaw cell viability.

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

The Petaka ™ is unique in that it is possible to measure the dissolved oxygen (pO_2) in your culture in real time, using a sterile, off-the-shelf setup. The Petaka ™ 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 ™ DO Sense it is easy to perform simple bioprocess monitoring or to carry out sophisticated experiments determining single cell oxygen consumption rates.

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To set up, simply fill the Petaka ™ DO Sense with your cell culture and incubate on any 37°C warming surface. This is only possible in the Petaka ™ because every other culture device requires a humidified CO_2 incubator. After filling, position the NeoFox™ reader to align with the sensing dot and start measuring. The user will then observe the classic pO_2 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 for days since there is practically no media loss due to evaporation and the pH of the culture is perfectly maintained without supplemental CO_2.

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

The Petaka ™ 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 ™ is designed and manufactured to withstand up to 650 g during routine centrifugation. Therefore, instead of aspirating and transferring cells for centrifugation, the user can simply place the Petaka ™ into the centrifuge and spin directly. This results in a cell pellet in the bottom corner of the Petaka ™. These cells stay pelleted as old media is aspirated off and fresh media added. Likewise, the pelleted cells may be selectively harvested while the old media is retained. This eliminates sample transfers into and out of centrifuge tubes, reducing contamination risk.

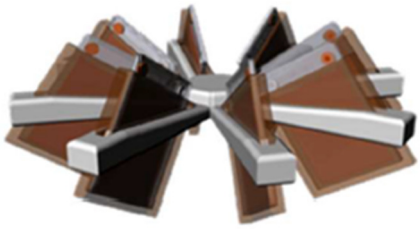

Figure 5. Centrifugation in the Petaka ™ rotor.

Why have my cells adhered to only one side of the Petaka ™?

In order to use the Petaka ™ for adherent cells and have the cells adhere to both surfaces of the Petaka ™ it is necessary to seed one side, wait for the cells to adhere for up to 3 hours, depending on the cell type and then re-seed the Petaka with cells and flip the unit over to allow the newly seeded cells to adhere. To introduce the second set of cells, withdraw a little of the media from the Petaka ™ to suspend the cells before seeding.

What do the coloured ports on the Petaka ™ indicate?

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

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

Standard plates, dishes and flasks must be open to allow open CO_2 gas exchange. The Petaka ™ is functionally closed and retains CO_2 in culture automatically. All liquid transfers are performed through a closed, sterile silicone port. An additional benefit is a reduced contamination risk during processing and the closed design means that media evaporation is practically eliminated. As such, there is no need to maintain a water tray inside the incubator, eliminating a major contamination source.

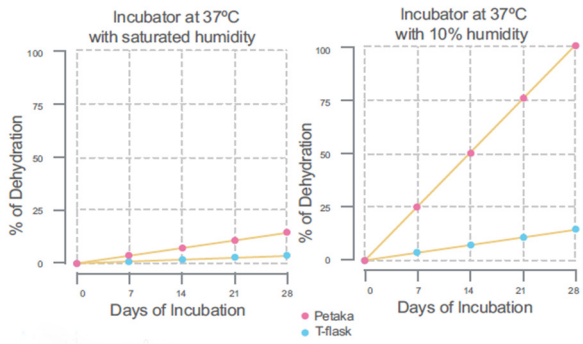

Figure 7. Showing dehydration rate over 28 day incubation period.

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

How can I ship cells in the Petaka ™?

Cells may be shipped at ambient temperatures using the Petaka ™. It is recommended that the Low Oxygen Units are used for this purpose. Cells should be seeded in the Petaka ™ unit 24 hours prior to shipping to allow them to acclimatise. The Low Oxygen Transfer unit is used since it is not ideal to have relatively high oxygen levels in the unit when shipping at temperatures below 37°C.

What is the capacity of cells in the Petaka ™?

The Petaka system is not designed for the production of tens of billions of cells. A typical suspension will produce 2.0-2.5MM cells/mL at peak density in Petaka ™, or 50MM cells per Petaka.

The Petaka ™ is ideal for higher quality and safer seed culture production, or for storage and transfer of samples for cryopreservation or downstream QC testing.

What are the basic features of the Petaka ™?

- Class VI medical grade resin
- Optically clear polystyrene growth surface
- Sterile (SAL 10-6)
- Standard SBS footprint
- 0.2 µm PTFE air filter

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- 24 m filling volume
- 95 kPa tested
- Free of detectable endotoxin
- Sterile silicone port access
- 75 cm^2 surface (one-sided culture)
- 150 cm surface (double-sided culture)
- Functionally closed processing
- Practically no media loss due to evaporation
- Single-use disposable

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