# PetakaG3™

## USER'S GUIDE

### Compact Cell Culture System Independent of $CO_2$ and Humidity

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*life in it!*

PetakaG3™

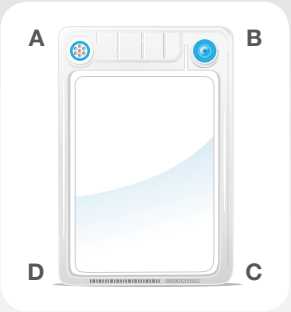
# A) Petaka's Basic traits

*Adherent and non-adherent cell culture*

Petaka is a cell culture device designed to culture adherent and nonadherent cells. Petaka is designed to eliminate all air space inside the culture chamber, which allows the anchorage and growth of adherent cells on all internal surfaces of the device.

## Petaka's shape description

Figure 1




Both air access (filter) and liquid access (port) are visible

Corner A: Is the corner where the air filter is located.
Corner B: Is the corner where the access port, septum, or diaphragm, is located.
Corner C: Is the corner where the internal gate to the chamber is located (I/O liquid flow).
Corner D: The most resistant corner to mechanical forces.

## No requirement of $CO_2$ incubator or humid incubator

Petaka is especially designed to grow cells in an isolated, automodified milieu, highly protected from external environmental conditions. That protection permits effective cell incubation with or without external CO2 control and independent of relative humidity in the atmosphere. A proprietary structural design establishes a gas exchange rate, which provides a suitable growing environment for the majority of cells incubated in regular atmosphere (0.2% $CO_2$ ). Additionally, this structure reduces dehydration to virtual rates, which allows the storage of media pre-loaded devices for weeks. Therefore, Petaka can be effectively incubated in any temperature-controlled chamber without $CO_2$ and humidity controlled environment.

## Exact volume of media per culture

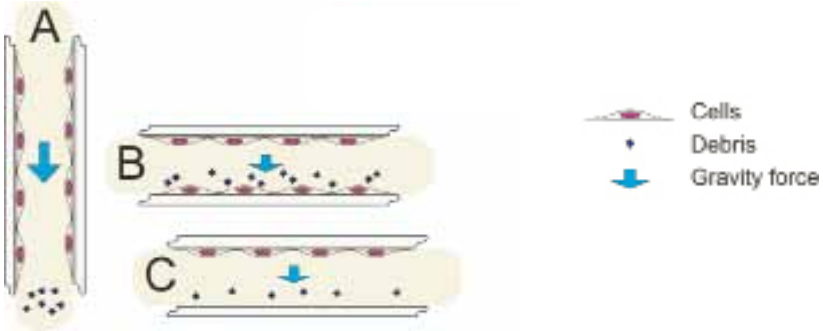
The internal cell culture chamber has 150 $cm^2$ of surface area available for cell attachment and growth, and 20 ml (+/-1%) of volume. This optimized media/surface area rate (0.133 ml/$cm^2$) facilitates the growth of variable millions of cells per Petaka, without media replacement, depending on the cellular metabolic and doubling time characteristics. Therefore, allowing optimal use of media and special additives, such as growth factors.

## Cell growth on vertical walls segregates cells and debris

Petaka offers a the unique feature of growing adherent cells on vertical walls, and on the ceiling surface; which permit the continuous segregation of growing cells and insoluble debris (Fig. 2). However, conventional horizontal cell growth is also suitable in this device.

Double sided adherent cell culture. (A) In vertical position the debris precipitates out of the cell surfaces. (B) In horizontal position, the upper layer is debris free, but the lower layer receives all debris. (C) Horizontal single side cell culture on the "ceiling" surface provides a solid debris reduced environment.

Figure 2



## Negligible dehydration rates

The structure of Petaka is designed to minimize the dehydration rate.
This permits elimination of the water pan from incubators, which can be set at RH = 5% - 30% (regular environment) without jeopardizing the cell culture. This also reduces the cross contamination risk in the incubator. Basically, Petaka can be stored full of media at room temperature or in refrigeration (4°C) and environmental humidity without dangerous dehydration for several months. That trait also makes possible the introduction of electronic and optical devices and instruments without restrictions in the incubators.

## Internal chamber never exposed to the external atmosphere

A special vent permits media injection and withdrawal without drastic compression and decompression forces inside the cell culture chamber (Fig 3 Exhaust). The vent is an air in and out access with airflow of about 1ml/sec under injecting pressure. This allows easy and rapid handling in operations of cell seeding, cell retrieval, media exchange etc. That air is sterility secured with a 0.2µm pore size filter. This prevents cell culture contamination by incoming air, and environment contamination by outgoing air.

Usual posture for holding Petaka during operations. One hand holds Petaka in quasi vertical position. The air exit is located in the uppermost corner of the culture chamber. To avoid liquids going into that exhaust gate is important. If the liquid goes into the exhaust the filter will be temporarily blocked (unblocking the filter will be required for continuing the normal procedure; see “filter unblocking procedures”)

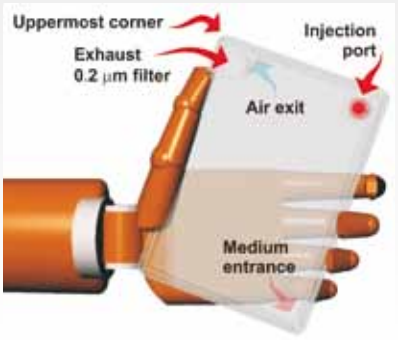



Figure 3

## No caps or lids

The media is introduced and withdraw from Petaka by injecting it through a port (an auto resealed diaphragm) into an atrium (Fig. 4 injection port). The atrium is connected to a 120 ml buffer channel (media channel) that ends in the culture chamber (Fig 4 media entrance). This channel, having only 1 $mm^2$ of diffusion interface with the main chamber, permits additives embedded in slow delivery media, to be gradually mixed in the main culture chamber.

## Protected against leaks or spills

Petaka has, integrated into its frame, a microfluidic head pressure trapping system interposed between the cell culture chamber and the air filter (Fig 5).
This trapping system avoid leaks or spills in any position or movement subjected by the users to the device (shaking, tapping, transportation, etc). This provides easy, comfortable & safe transportation. Leaks are prevented even when Petaka is subjected to unusual pressures, movements, knocks and orientations.

Figure 4

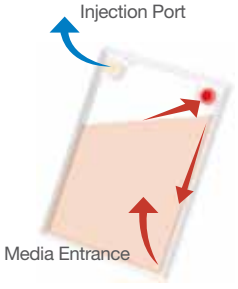




Figure 5

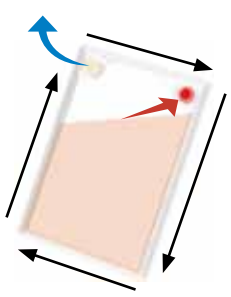


Figure 6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# B) Cell culture in Petaka. Instructions of use

## B.1.- Sterility Produres

Petaka is delivered gamma-sterilized.
Petaka cannot be autoclaved!

### B.1.1.- Cleaning parts of Petaka, and tips


Figure 7

**Global wash.** Petaka can be externally washed (if needed) with ethanol or detergents dissolved in water. However, the air exhaust should be protected from liquids.
**Injection port.** The port diaphragm is the most critical point for sterility precautions, while the tips will contact and cross it. Immediately before and after use, the port surface should be dried and thoroughly swabbed with 90% ethanol (Fig. 7).
**Liquid handling tips:** For every liquid transfer operation, new sterile liquid-handling tips should be used. Metallic tips may be used repeatedly, slightly flaming them (with a Bunsen, alcohol flame, or a cigarette lighter) before contacting the diaphragm surface.

**WARNING**

If the air exhaust is not properly protected, during washing, the filter could be damaged and normal Petaka's function impaired.

## B.2. - Introducing media and cells into Petaka.

### B.2.1 Going through the port diaphragm (tip insertion).

Media, additives and cell suspension are introduced in Petaka though the port, using any kind of available liquid handling tip and device (Figs. 4, 8 & 9).

### B.2.1.1 When Petaka is new (first insertion)

Figure 8

Use an 18 gage metallic tip to perforate the port diaphragm initially (Fig 8). Apply the tip on the diaphragm surface and press it until the tip penetrates the diaphragm entirely.. Normally a characteristic "click" is audible.

Be sure the tip actually penetrates the diaphragm!

### B.2.1.2 When Petaka has been used (after first insertion)

After the first insertion any kind of liquid dispensing tip, plastic or metallic, with an external end diameter smaller than 1.5 mm, is acceptable.
Be sure the tip actually penetrates the diaphragm!

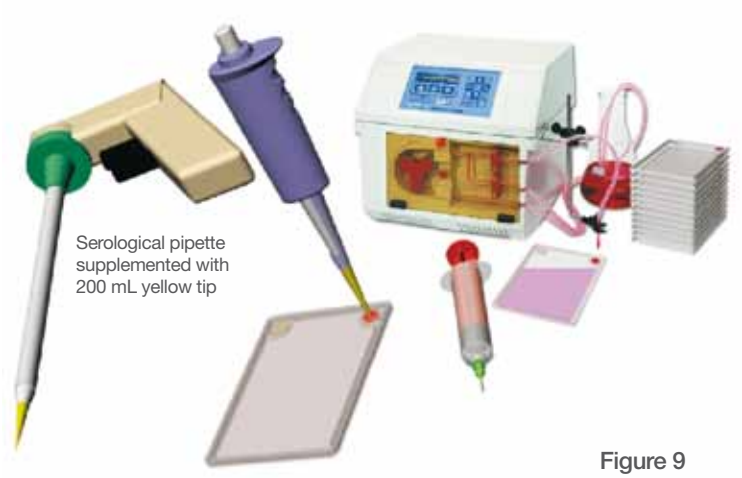



Figure 9

## B.2.2. Filling an empty Petaka.

The total capacity of the Petaka culture chamber is 20 ml. For filling the major volume of media a simple 30 ml syringe is recommended or, if available, repetitive dispensers (for example: peristaltic dispensers, or programmable peristaltic pumps) could be really useful for large-scale works (Fig 9).

Figure 10

The recommended posture for injecting media is:
Holding Petaka with one hand, maintaining the air exhausts on the uppermost position, and injecting the media with the other hand (Fig 10).
Injection of full 20 ml should be performed slowly, in about 5 seconds. Due to the airflow resistance of the filter, the device will slightly expand and the air space
on top of the media will be significant after 20-22ml injection, building certain internal positive pressure.

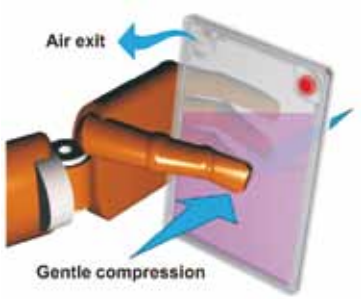



Figure 11

A soft squishing action, with the fingers (Fig 11), will accelerate the airflow through the filter and rapidly eliminate the air excess and the internal positive pressure.
Do not allow liquid to reach the air exhaust when there is an internal positive pressure. After injecting 20-22ml of media, watchfully squish the device to eliminate the excess of air through the exhaust (Fig 11).

The device will indicate the absence of internal positive pressure when the air back flows into the chamber through the exhaust, after releasing the squishing force.

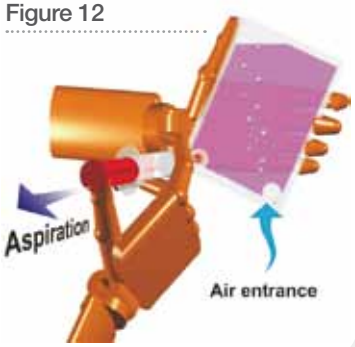
**WARNING**

1. Only when internal pressure is purged can Petaka be placed in horizontal position.
2. If media enters the microfluidic trap accidentally, and reaches the filter, inflow will be blocked, producing a high resistance to injection.
3. If that happens, perform a “flow de-blocking procedure” (see B.2.3.) and continue with the filling process.
4. Raising the pressure to override increased flow resistance, if the media went into the exhaust accidentally, will force the media though the filter and damage it. Thus, Petaka will be ruined.

### B.2.3 - Flow deblocking (FDP) procedure.

If media accidentally enters into the microfluid trap during a liquid injection procedure accidentally, and reaches the filter, the inflow will be blocked, offering a high resistance to injection. To remedy this:

Figure 12




a) Place the Petaka upside down (Fig.12)
b) Withdraw 7-to10 ml of fluid (air or media) from the internal chamber and hold Petaka in the same position.
c) After a couple of seconds, a babbling airflow will appear emerging from the exhaust. That indicates that the filter and microfluidic trap are liquid free, and the normal flow reestablished.
d) Continue the filling operation in the correct posture (Fig 10).

This operation can be repeated any time an accident blocks the exhaust.

### B.2.4. Adding fluid into a partially full Petaka .

Additional fluids can be introduced in Petaka with any kind of syringe, pipette, etc. Any addition of fluid (serum, growth factors, etc.) into the culture chamber requires setting the Petaka in a vertical position, and occasionally, emptying the microfluidic trap if full by performing an media withdraw of 7 mL of media and holding it in the syringe for about 5 seconds before reinjection.
After fluid addition expel the excess of air with a gentle compression of Petaka with the fingertips, as described above (B.2.2, B.2.3, and Figs 10 & 11).

**Remember**

The maximum volume accepted in Petaka is 20 ml.

### B.2.5. Adding cells into a partially full Petaka

Any addition of cells suspended in more than 2 mL of fluid into the culture chamber, requires setting Petaka in a vertical position and emptying the microfluidic trap as described above.

**Remember**

The maximum volume accepted in Petaka is 20 ml. After the cell suspension addition, expel excess air with a gentle compression of Petaka with the fingertips, as described above (B.2.2 and Figs 10 & 11).

## B.3. Surveillance of the cell attachment process

Cell attachment can be monitored as usual, with any kind of light microscope. While the maximum thickness of Petaka is only 5.2 mm either inverted or regular microscopes can be used depending on the condenser specifications.
The thickness of the walls is 0.9mm; therefore, all 6x, 10x, 20x, 25x and a majority of 40x dry objectives can be used. However, short distance, immersion, 63x, and 100x objectives cannot be used.

### B.3.1 Cell attachment surveillance for regular “both sides” cell culture

Surveillance of cell attachment is important for efficiently growing cells on both sides of Petaka.
After introduction of media and cells it is important to warrant the absence of positive pressure inside the culture chamber as explained before (B.2.2).
When the internal pressure is balanced, Petaka should be placed in the incubator horizontally, to allow cell attachment on the bottom surface of the chamber.

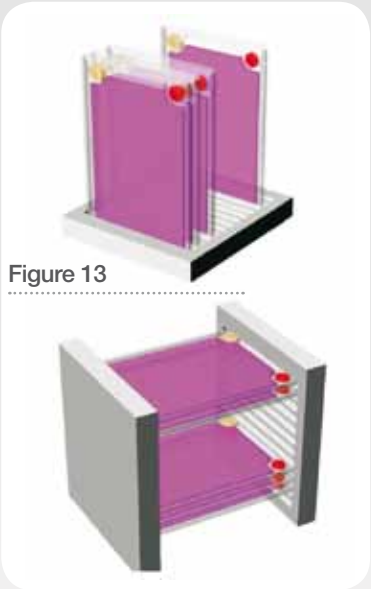


Figure 13

For cells with an unfamiliar behaviour, controlling the initial cell attachment every 10 minutes is recommended. When approximately 30-50% of the cells become attached (first period of attachment), Petaka should be flipped horizontally (optimal flipping moment), and incubated in the new position for at least 3 hours, or twice the time of the first period of attachment; that will allow contact and attachment of the remaining floating cells to the opposite side.
After that period of incubation (final period of attachment), cells will be attached on both sides. Then Petaka can be incubated in any possible orientation (vertical or horizontal, Fig 13). Cells will grow attached either to the upper surface or the lower surface. Likewise, after attachment, cells will grow on vertical surfaces.

Petaka, like T-flasks, multi-well plates, dishes, etc, is made of polystyrene. A majority of the usual adherent cell lines achieve full attachment to uncoated polystyrene surface in 10 to 120 minutes. That variability is associated with cell type, cell preservation, media, environment status, age of the cell culture device, etc. Therefore, the investigation and determination of the optimal flipping moment for each case is highly recommended.

## B.4. Temperature, $CO_2$ $O_2$ and humidity needs.

Petaka has an internal closed environment with practically no $CO_2$ loses, which allows growing cell cultures to be maintained in very low $CO_2$ concentrations (regular atmospheric concentration) for long periods of time without cell harm. However, Petaka may also be incubated in regular incubators at 37.5 ºC and controlled $CO_2$ concentration (5-10%).
Likewise, the $O_2$ diffusion from the outside atmosphere into the culture chamber is quenched by the microfluidic system, and the specific oxygen permeability of the Petaka's polymer, creating a suitable environment in the cell culture chamber. The system is calculated to provide a finite time suitable oxygen concentration for rapidly growing cells and 100% confluent cultures.
Most eukaryotic cells grow perfectly in this environment. However, cells with particularly highly oxidative metabolism could consume oxygen at faster rate than the deliberate exchange rate of the microfluidic system, thus limiting the yield (Appendix A). These cells will need more frequent media exchange or "gassing" maneuvers (see "Gassing the media"). Alternatively, these cells could be cultured vertically in only 20 ml of media, leaving a 5ml air bubble on top, directly in continuity with the air exhaust. This way of culturing cells may require a 5-10%$CO_2$ atmosphere.
The microfluidic system reduces the water evaporation in Petaka to minimal rates. That permits culturing cells at 37.5 ºC with a relative humidity (RH) of 10-30% inside the incubator, which is practically the same RH as a regular environment of a dry incubator (Fig. 14).

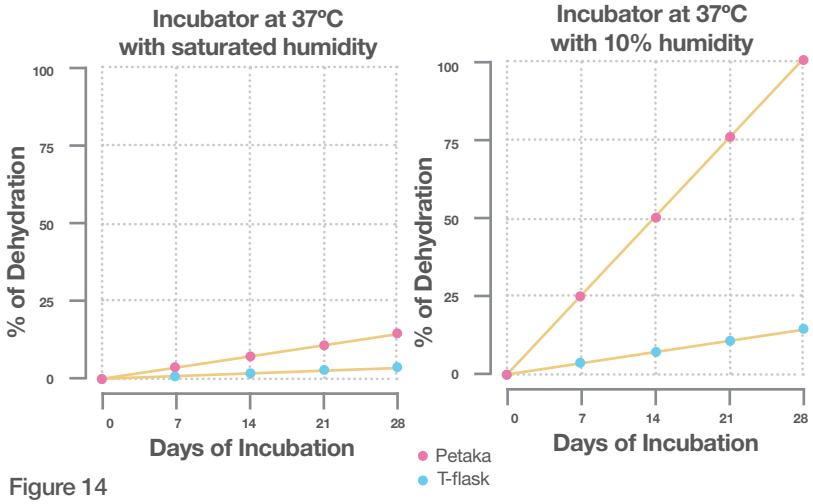



Figure 14

At 37.5ºC the vi r tual hermetical structure of Petaka controls the dehydration rate in very dry environment (10% RH) below the limits of the T75 flasks in humid incubators (>90% RH).
At 22ºC Petaka holds the dehydration rate, in very dry environment (10% RH), below 0.5 mL per month..

## B.5. - Monitoring the cell growth

Degrees of cell confluence can be assessed, like in any other common cell culture device, by observations with phase contrast microscopy.
With 10x to 25x long distance phase contrast objectives, cells on both sides of Petaka can be counted without flipping it.

## B.6. Replacing media when needed

The media exchange operation of attached cells can be performed with syringe and tip. The 20 ml of old media can be removed with the syringe or pipette, slowly to permit airflow into the culture chamber via the filter. The internal low pressure pressure produced by the media extraction will be automatically compensated by a minor depression of the Petaka walls. The wall depression can be monitored through the thickness of the media layer in the center of Petaka. Do not allow contact of the opposite walls. Slow down the media extraction if the media layer becomes too thin.
For media replacement follow the same steps described in section B.2.2. (Filling an empty Petaka)
Non-attached cells need to be concentrated against a corner before the extraction of the old media. Cells can be concentrated in a corner of
Petaka by centrifugation in a special rotor (see “Petaka centrifugation”). After cell concentration, media extraction is performed as above. Cells will be resuspended after media replacement.

**Remember**

The maximum volume accepted in Petaka is 20 ml.
After media replacement expel the excess of air with a gentle compression of Petaka with the fingertips, as described above (B.2.2 and Fig 7).

## B.7. Harvesting cell culture products

Many particular features differentiate Petaka from other cell culture devices. One outstanding difference is the fact that Petaka permits cell concentration by centrifugation in the same device , avoiding intermediate cell transfers to centrifuge tubes.

### B.7.1 Harvesting non-adherent cells.

Non-attached cells can be concentrated against corner C before harvesting (Fig. 1). Cells can be concentrated in corner C of Petaka by centrifugation in a special rotor (see “Petaka centrifugation”), or by gravity, holding Petaka vertically with corner C in the lowermost position for a user-defined period of time (overnight is enough for SP2 myeloma cells as an example). After concentration, cells can be harvested by aspirating the sediment together with the desired volume of additional medium. Alternatively, cells could be extracted with the media and concentrated in a conical tube as traditionally done.

### B.7.2 Harvesting media from nonadherent cells.

Media of non-attached cells can be harvested by first concentrating the cells in corner D (Fig 1). Cells can be concentrated in this corner by centrifugation in a special rotor (see “Petaka centrifugation”), or by gravity, holding Petaka in a vertical position, with corner D in the lowermost point for a user defined period of time (overnight is enough for SP2 myeloma cells as an example) (Fig. 1). After cell concentration, the medium can be harvested by aspirating the desired volume.

### B.7.3 Harvesting adherent cells.

Adherent cells need to be detached before harvesting. A standard protocol should be applied as follow:

a) Remove all media while holding Petaka in a vertical position to avoid foam formation from the incoming air.
b) Introduce 3-4 mL of dissociation enzymes solution (example: 0.25% Trypsin / 0.1% EDTA).

c) Gently shake Petaka until the enzyme solution wets the entire internal surfaces.
d) Incubate for 1-2 minutes at 37ºC or 3 min at room temperature.
e) Confirm cell detachment under the microscope.
f) Tap Petaka a couple of times and leave it vertical a few seconds to allow the solution to collect on the bottom.
g) Remove the cell suspension and proceed with the next protocol

**Remember**

Suspensions of cells in enzyme solution may show a certain viscosity, which could provoke the retention of a significant amount of clumped cells in droplets attached to the walls. If that happens, after the extraction of the enzyme solution, introduce 3-5 mL of media or PBS, gently shake Petaka and extract that cell suspension.

### 7.4 Harvesting media from adherent cells.

Media of attached cells can be harvested through a simple aspiration.
The harvesting operation can be performed with syringe and tip. The 20 mL of conditioned media should be removed slowly to permit air entrance into the culture chamber. The internal low pressure produced by the media extraction will be compensated by an automatic minor depression of the Petaka walls. The wall depression can be monitored through the thickness of the media layer in the center of Petaka. Do not allow the contact of opposite walls. Slow down the extraction if the media layer becomes too thin.

**Remember**

An extremely rapid extraction, which would produce the collapse and contact of both walls of Petaka, would provoke the death of the cells covering the contacting surfaces.

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# C) "Ready to Host" Petakas (RHPs)use

## C.1 Preparation and Storage of RHPs.

Petaka has a highly insulated milieu, protected from the external environment. That protection, together with the optimized volume permits filling with media and additives, and conveniently storing them before injecting the cells. So, a convenient number of Petaka can be prepared with specific media and additives and stored in the refrigerator "ready to host" cells (Ready to Host Petaka RHP-). Dehydration is virtually zero up to 120 days at 4ºC. However, wrapping groups of Petakas within plastic zip-bag is recommended to guarantee full preservation of the content, because humidity conditions differ in refrigerators.
Therefore, storage time will be limited only by the properties of the media components and additives (fetal bovine serum, antibiotics, etc).
RHPs should be stored in the refrigerator always vertically.
Transfer of RHPs, from the refrigerator to the incubator, should be made 20 minutes before the injection of cell suspensions.

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# D) Cell culture "Dormancy"

## D.1 A unique ability of Petaka.

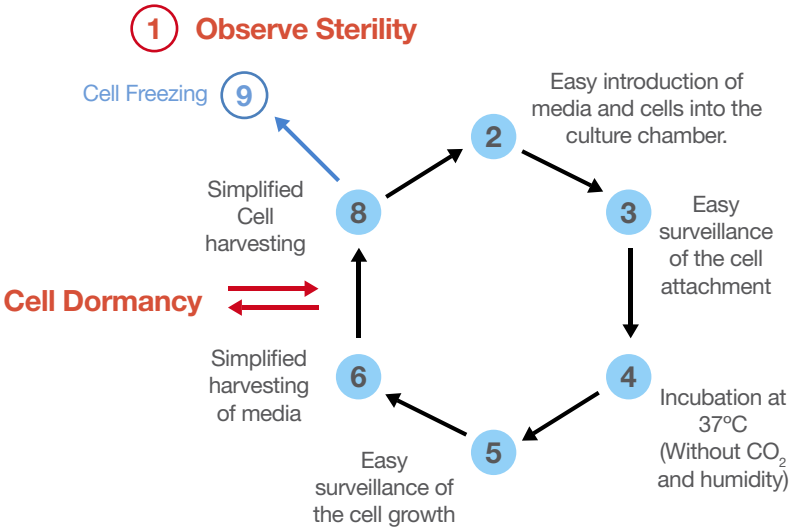
The highly insulated, highly protected milieu of Petaka allows the storage of cell cultures at room temperature and environment for extended periods of time, depending on the cell type, the media, and the culture status. The storage of cultured Petakas at room temperature are not susceptible to dehydration for months (up to 3 months at 22ºC and 25% humidity).
After Dormancy, cells returned to the incubator will continue growing after a short LAG period. Alternatively, after dormancy the cultures could be directly expanded into new Petakas.
This unique ability of Petaka can be used for:

- Avoiding media exchanges exclusively forced by culture maintenance requirement.
- To put cultures on hold before freezing.
- Holding cultures for schedule purposes.
- Transportation of cell cultures.
- Others.

Due to the photosensitivity associated to same types of buffers (HEPES) and cell types, it is recommended to store the cell cultures in the dark. ).

## D.2 In vitro cell dormancy, a powerful new step in cell culture

Cell culture in Petaka fulfils every step of the cell culture process and incorporates the unique ability of storing the cells at room temperature for extended periods of time.



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# E) Yields

Petaka has 150 $cm^2$ of surface available for cell attachment, and 20.0 mL capacity for media.
The number of cells obtainable from a single Petaka has an upper limit established by the available surface area (theoretically about 200,000 cells x150 cm = 30,000,000 cells). The experimental average yield (tested in laboratories) is about 750,000 cells/mL of media, equal or slightly better* than the average yields of the classical T-Flask (600,000 cells/mL of media)
Both the volume of media and the gas exchange rate, distinctive of Petaka technology, may impose other limits depending on the cultures cell type and the media used. Mainly, these limits will be associated with: The number of cells introduced as seed, the typical oxygen consumed per minute per cell of each specific type, and the doubling time of each specific cell type.
As an orientation, appendix A shows tables of yields constructed by computer simulation based on two hypothetical cell types: a) A hypothetical cell type consuming 0.4 fempto-Moles of oxygen per minute per cell (fM/min/cell), and b) a second hypothetical cell type consuming 0.2 fM/min/cell of oxygen. Both cell types are supposed to be growing in media buffered with bicarbonate, with 4 g/L of glucose and pyruvate.
The tables show that pH may be a limiting factor (yellow and orange boundaries), and hypoxia as well (purple boundaries). A change of media may override these limits.

*(*) This will depend on the cell type and medium used. Some cell types could be limited in number due to the $CO_2$ production*

**Remember**

Cells in Petaka do not behave exactly like in flasks or other devices.
First time a cell type is grown in Petaka, an optimization testing is highly recommended.

# APPENDIX A

PETAKA yields (Millions of cells) depending on Oxygen consumption per cell, number of cells seeded, and doubling time. (Computer simulation)

Oxygen consumption per cell

| | 0.4 fM/min | | | | | 0.2 fM/min | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Doubling Time | | | | | Doubling Time | | | | |
| Seed 100, 000 Cells | 18 h | 20 h | 22 h | 24 h | 26 h | 18 h | 20 h | 22 h | 24 h | 26 h |
| Days of culture | Average | Average | Average | Average | Average | Average | Average | Average | Average | Average |
| 0 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| 1 | 0.21 | 0.20 | 0.18 | 0.18 | 0.17 | 0.21 | 0.20 | 0.18 | 0.18 | 0.17 |
| 2 | 0.46 | 0.39 | 0.35 | 0.31 | 0.29 | 0.46 | 0.39 | 0.35 | 0.31 | 0.29 |
| 3 | 0.99 | 0.79 | 0.65 | 0.56 | 0.49 | 0.99 | 0.79 | 0.65 | 0.56 | 0.49 |
| 4 | 2.14 | 1.57 | 1.22 | 0.99 | 0.83 | 2.14 | 1.57 | 1.22 | 0.99 | 0.83 |
| 5 | 5.19 | 3.15 | 2.30 | 1.77 | 1.41 | 5.19 | 3.15 | 2.30 | 1.77 | 1.41 |
| 6 | 13.03 | 7.23 | 4.73 | 3.30 | 2.41 | 13.03 | 7.23 | 4.73 | 3.30 | 2.41 |
| 7 | 23.33 | 16.24 | 10.08 | 6.59 | 4.44 | 23.33 | 16.24 | 10.08 | 6.59 | 4.44 |
| 8 | 34.08 | 26.81 | 19.68 | 13.19 | 8.43 | 34.08 | 26.81 | 19.68 | 13.19 | 8.43 |
| 9 | 44.45 | 37.53 | 30.38 | 23.51 | 16.29 | 44.45 | 37.53 | 30.38 | 23.51 | 16.29 |
| 10 | 44.45 | 37.53 | 40.96 | 34.26 | 26.83 | 44.45 | 37.53 | 40.96 | 34.26 | 26.83 |
| Seed 1,000,000 Cells | | | | | | | | | | |
| 0 | 0.98 | 0.98 | 0.98 | 0.98 | 0.98 | 0.98 | 0.98 | 0.98 | 0.98 | 0.98 |
| 1 | 2.12 | 1.97 | 1.85 | 1.75 | 1.68 | 2.12 | 1.97 | 1.85 | 1.75 | 1.68 |
| 2 | 5.15 | 4.36 | 3.65 | 3.27 | 2.86 | 5.15 | 4.36 | 3.65 | 3.27 | 2.86 |
| 3 | 12.95 | 10.02 | 7.77 | 6.54 | 5.42 | 12.95 | 10.02 | 7.77 | 6.54 | 5.42 |
| 4 | 23.24 | 20.00 | 16.19 | 13.08 | 10.27 | 23.24 | 20.00 | 16.19 | 13.08 | 10.27 |
| 5 | 33.99 | 30.72 | 26.74 | 23.39 | 19.53 | 33.99 | 30.72 | 26.74 | 23.39 | 19.53 |
| 6 | 44.37 | 41.29 | 37.44 | 34.15 | 30.23 | 44.37 | 41.29 | 37.44 | 34.15 | 30.23 |
| 7 | 44.37 | 41.29 | 37.44 | 44.52 | 40.82 | 44.37 | 41.29 | 37.44 | 44.52 | 40.82 |
| 8 | | | | 44.52 | 40.82 | | | | 44.52 | 40.82 |
| Seed 2,000,000 Cells | | | | | | | | | | |
| 0 | 1.97 | 1.97 | 1.97 | 1.97 | 1.97 | 1.97 | 1.97 | 1.97 | 1.97 | 1.97 |
| 1 | 4.76 | 4.36 | 4.05 | 3.82 | 3.63 | 4.76 | 4.36 | 4.05 | 3.82 | 3.63 |
| 2 | 12.00 | 10.02 | 8.64 | 7.63 | 6.88 | 12.00 | 10.02 | 8.64 | 7.63 | 6.88 |
| 3 | 22.21 | 20.00 | 17.69 | 15.50 | 13.04 | 22.21 | 20.00 | 17.69 | 15.50 | 13.04 |
| 4 | 32.96 | 30.72 | 28.31 | 25.99 | 23.35 | 32.96 | 30.72 | 28.31 | 25.99 | 23.35 |
| 5 | 43.40 | 41.29 | 38.97 | 36.70 | 34.10 | 43.40 | 41.29 | 38.97 | 36.70 | 34.10 |
| 6 | 43.40 | 41.29 | 38.97 | 36.70 | 44.48 | 43.40 | 41.29 | 38.97 | 36.70 | 44.48 |

- pH balanced, O2 = 6-7 mg/L
- pH compromising the culture
- pH damaging the culture (cellular stress)
- pH stops cell growth and some cells die
- Hypoxic environment O2<1mg/L

# APPENDIX B

Basic Specifications

## General characteristics

| | |
|---|---|
| Disposable device | Single use |
| Footprint | Microtiter plate |
| Color | Clear |
| Cell growth surface area | Nominal 150 $cm^2$ |
| Volume of Media l | 20 ml |
| Air space | < 1.5 ml |
| Total space occupied per unit | 53 $cm^3$ |
| Total weight without media | 32,000 mg |
| Total weight with media | 52,000 mg |
| Average density without media | 1.05 |
| Integrated unit identifier | Individual Barcode |
| Material | Crystal Polystyrene |

## Dimensions

| | |
|---|---|
| X | 127.5 mm + /- 0.5 mm |
| Y | 85.5 mm + /- 0.5 mm |
| Z | 5 mm + /- 0.2 mm |

## Cell growth surface

| | |
|---|---|
| Type of growth surface | Crystal Polystyrene |
| Growth Surface thickness | 0.9 mm |
| Growth surface color | Clear |
| Space bet ween growth surfaces | 3 mm |
| Ext ernal surface tension (energy) | 32-38 dynes/ cm |
| Internal surface tension (energy) (Corona treated) | 50-64 dynes/ cm |

## Vent properties

| | |
|---|---|
| Spontaneous dehydration | |
| 38ºC and 90% Humidity | 0.014 ± 0.005 m l/day |
| 38ºC and 10% Humidity | 0.14 ± 0.01 m l/day |
| 22ºC and 50% Humidity | 0.001 ± 0.0001 m l/day |

## Access Port

| | |
|---|---|
| Number Access ports | 1 |
| Shape | Circular |
| Color | Orange (Pantone 021U) |
| Size | OD 6 mm |
| Port mat erial | Biocomcompatible, Non-cytotoxic elastomer Exemempt of free radicals, surfactants, detergents organic solvents, pyrogenic components |
| Surface Disinfectable with | Alcohol, Benzalkonium chloride, etc. |
| I/O Access device | 16-20 gage blunt canulae/tips (ID up to 1 mm) |
| Atrium volume | < 50 µL |
| Buffer reservoir volume | 80-100 µL |
| Port operation | Press ure penetration, auto-sealed |

## Optical properties

| | |
|---|---|
| Refraction Index | 1.57 |
| Light Absorption Spectrum | From 310 nm t o 670 nm absorbanc e < 1% |
| Autofluorescence wl | < 485 nm |
| Admitted optical resolution | 0.25 µm |

## Petaka sterilization

| | |
|---|---|
| Factory packaged | Gamma sterilized |
| After first use | Not autoc lavable |
| Sterilization tolerance | Admits ETO, UV, and Gamma radiation |