

PetakaG3™

the most advanced
cell culture device
available in the
market today

www.petaka.com
life in it!



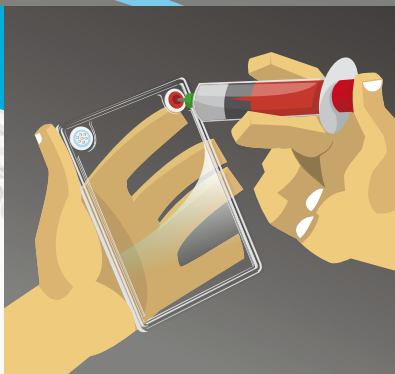
PetakaG3™

new and superior technology

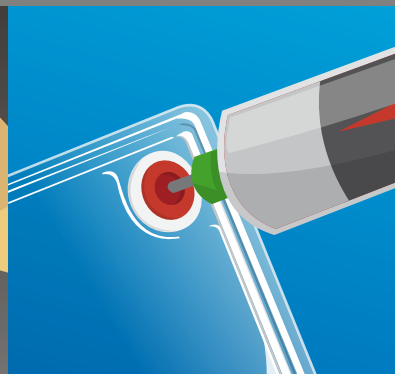
A single step
to scientific cell culture



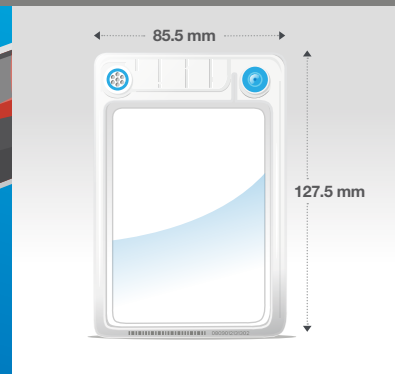
Virtually Hermetic Enclosed™



Gas transfer Quenching system™



In-situ media/cell segregation™



Petaka's user friendly design

Virtually Hermetic Enclosed™ (“VHES”)

Eliminates direct contact with the atmosphere. Therefore, reduces maneuver risk, practically eradicates culture contamination and cross contamination, removes shear forces (avoids media displacement, media waiving and bubbling) and gets rid of any kind of spills or leaks.

Minimum Static™ (“MSM”)

Optimizes both, the ratio media/cellular yield, and the need for growth factor. Accordingly, cell culture with Petaka is more cost effective.

In-Situ Media/Cell Segregation™ (“MCSS”)

Simplifies operations by performing two functions, cell growth and cell concentration, in a single device. As a result, cell culture becomes simpler and more versatile.

Gas Transfer Quenching System™ (“GTQS”)

Allows suitable gas exchange without gas bubbling, media steering, media perfusion, or gas permeable membranes. Thanks to this system neither CO₂, nor saturated humidity is required to grow cells. This enables safe handling as well as a reduction in turnaround time and overall equipment cost.

Petaka's design

Is user friendly in many ways. Its resealable access port, which keeps the interior hermetic to the exterior elements, combined with the gas transfer quenching system and the filtered exit vent, allows for a safe (no contact with the atmosphere, no saturated humidity in the incubator) and simple one-step-access-or-retrieval operation. In addition, safe handling minimizes the number of routines thus basically reducing cell culture cost.

Petaka's design has managed to offer 150 cm² of growth surface with only 20 ml. of media, and 127.5 x 85.5 x 5 mm. external dimensions. Its shape and size makes it comfortable and easy to handle, efficient to store and trouble-free and safe to transport, all in a cost-effective fashion.

PetakaG3™

compared to any other cell culture device

- No additional CO₂ required for any type of media, although the PetakaG3™ device can also be used in CO₂ incubators
- No added humidity required (not influenced by environmental dehydration)
- The most secure cell culture device against contamination available today (no caps, no openings, no spills)
- Robust structure (without breakable films)
- Excellent for shipping live cells without requiring freezing and dry ice
- Optimizes media and growth factor supplies
- Produces 8 times more media volume per incubator capacity
- Provides 15 times more cell culture surface per incubator capacity
- In vitro cell dormancy is easily accomplished
- Allows storage of pre-filled units that are ready to host cells
- Easy to handle
- Operates in any position



PetakaG3™

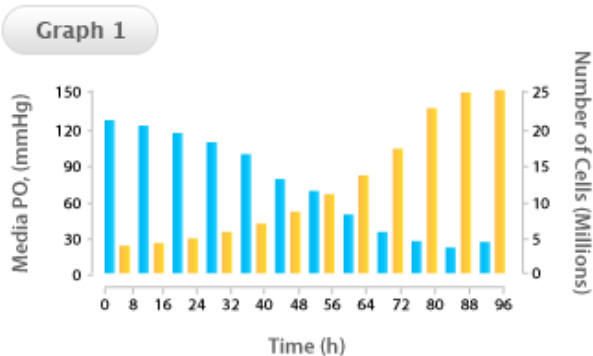
oxygen concentrations

Achieving the correct concentration is no longer a problem design

Petaka provides the cells with a gradually decreasing oxygen concentration in the media, within the physiological limits of living tissues (Graph 1).

Cell culture may begin with a concentration equivalent to that of arterial blood (OP 75 mmHg), high enough to promote exponential cell growth. The concentration is then progressively reduced to that of embryonic development (OP 15 mmHg), avoiding cell damage and facilitating cell differentiation.

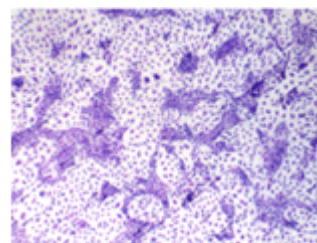
Average evolution of Oxygen concentration (blue) & cell growth in Petaka (yellow)



BASICS

Oxygen is the basic element for the cell's aerobic respiration, which is a highly efficient energy source. In its absence, energy is generated in the nucleated cell by anaerobic respiration, which is only 5% as efficient as aerobic.

Therefore, cultured cells need oxygen, but not in excess. When cells are cultured in media in classic cell culture devices, such as flasks and petri dishes, excessive oxygen is provided. In these devices, the media surface forms an interface with the atmosphere, which contains about 20% oxygen; through that interface, the oxygen in the air dissolves in the media up to the limit of its solubility in water, depending on cell culture temperature, atmospheric pressure and the degree of salinity of the media. This results in oxygen concentrations that are too high for normal cells, which naturally live in the lower oxygen environment found in living bodies



Breast cancer cells differentiated in Petaka

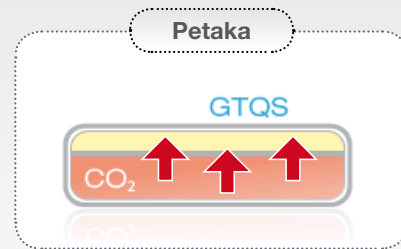
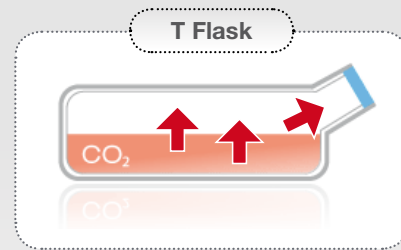
PetakaG3™

no need for CO₂

CO₂ cartridges are no longer needed

Petaka is designed to allow very low amounts of CO₂ in and out. Additional CO₂ is therefore not required, avoiding the need for CO₂ cartridges. CO₂ produced by the cell culture is also controlled. This is achieved by a gas transfer quenching system (GTQS) integrated in the device. As a result the level of CO₂ is maintained within the Petaka, allowing the pH to remain at a level compatible with the growth of 16 to 22 million cells.

Therefore, Petaka can be incubated in normal incubators without CO₂, and cells grow in a closed and stable environment up to the limit of their own metabolism. These yields will vary according to each individual cell metabolism. We recommend that a culture test and a period of adaptation are implemented when a cell line is going to be grown in Petaka.



BASICS

Media based on Earle's salts are buffered with a bicarbonate/carbonic acid system. These buffers rely upon the physiologically relevant pKa for carbonic acid and the equilibration of gaseous and media dissolved carbon dioxide to maintain the pH in a CO₂ equilibrated incubator. Use of Earle's salts in classic cell culture devices, but not in Petaka, at atmospheric conditions (0.04% CO₂) will result in a rapid rise in pH of the culture medium. This will cause the phenol red indicator to turn purple and cells will die. This does not happen in Petaka.

PetakaG3™

hypoxia experiments

Hypoxia experiments without special incubators

Low oxygen concentration in the cell culture media has a significant impact on cell physiology and differentiation. Cells kept in traditional hyperoxic culture conditions provided by media in contact with ambient air containing 20 % oxygen behave very differently.

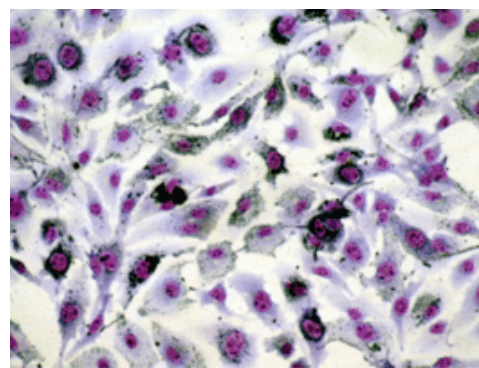
To research cellular behavior under hypoxic or normoxic conditions, laboratories use controlled-environment incubators and glove boxes which offer the possibility of working in below ambient O₂ concentration.

Controlled-environment incubators only allow the incubation of tissue culture flasks in a controlled oxygen atmospheres. Glove boxes offer researchers the ability to incubate or perform sample manipulations without compromising the chamber's gas-controlled environment. However the manipulation of cell cultures in glove boxes can be difficult and uncomfortable. Petaka does not need special incubators or glove boxes.

Petaka offers the researcher complete freedom of sample manipulation even in very low oxygen hypoxic conditions, without breaking the hypoxic environment at any time.

BASICS

Petaka, when packed in vacuum mylar bags, do not allow the passage of oxygen from the ambient atmosphere to the culture and the cells consume the media-dissolved oxygen in hours, creating a deep hypoxic condition. Cells can be grown and incubated in these conditions and the cell culture eventually withdrawn by punching the port directly through the mylar bag, avoiding any exposure to higher oxygen concentrations.



Melanoma cells cultured in deep hypoxia (<3% O₂) for 72 hours. Regular incubator and Petaka in vacuum mylar bags.

PetakaG3™

no humidifiers

No more water pan or humidifier inside the incubator

Petaka is virtually vapor hermetic

- Petaka does not require a humidity saturated incubator.
- You may store pre-filled Petakas, ready to host cells, and have them available for immediate use.

Increased protection against incubator cross contamination

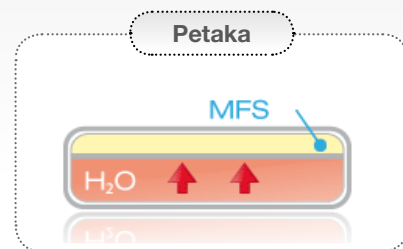
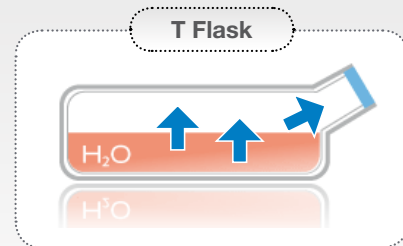
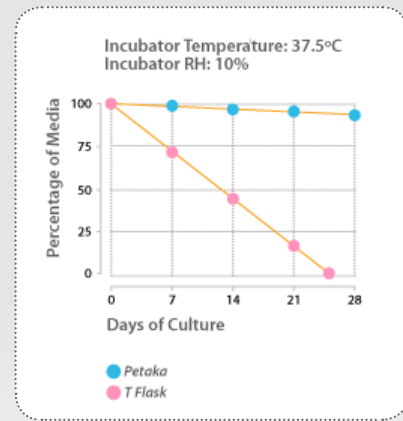
- Elimination of saturated humidity in the incubator, water pan, and mist, reduces the risk of cross contamination in the incubator.

Refrigerated storage time is not limited by dehydration

- Petaka pre-filled with media can be stored for a long time in most refrigerators at 4°C without the risk of dehydration. The refrigerator type should be considered before storage to avoid unexpected dehydration rates.

BASICS

In Petaka, an integrated micro-channel system protects the internal environment, avoiding unwanted water evaporation. Dehydration at 37°C has been minimized, allowing culture incubation for 30 days at RH 10% with a final media dehydration below 10%.



PetakaG3™

antiseptics

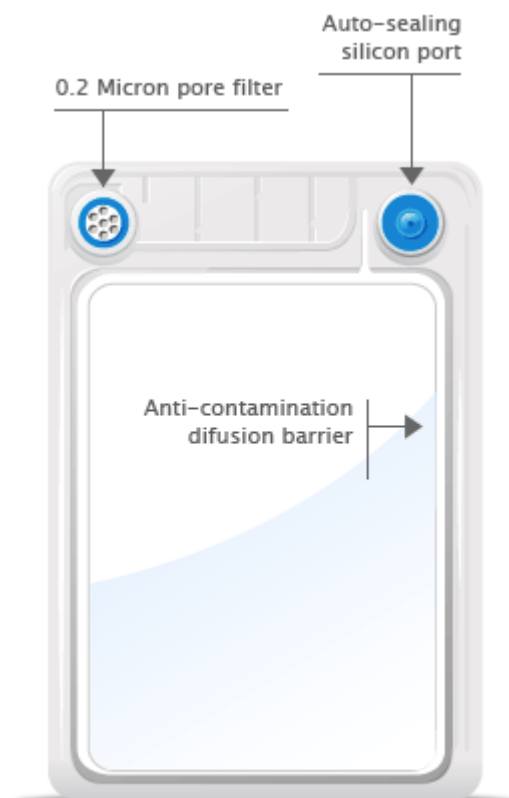
Microbial contamination is a severe problem in cell culture. Typical routes of microbial infection in cultures are the ambient air, when flasks are transferred from the hood to the incubator, the water bath, and the humid environment of the CO₂ incubator which provides ideal growing conditions for many strains of bacteria.

Other routes of infection include: contact with non-sterile surfaces when performing cell culture manipulations, spillage on materials and the work surface, splash-back from pipetting or pouring cell suspensions and microscopic aerosols.

Petaka is a virtually hermetically closed system. Air access is only possible through a 0.2 micron pore filter. Neither lids nor caps are used to access the liquids. Petaka are injected through a silicon port that seals after the removal of the injection tip.

Sterilizing the injection tips and the silicon port minimizes the chances of contamination. In addition, Petaka has an 80 mm diffusion barrier between the port and the culture chamber. This exponentially decreases the chances of microbial progression through the port slit during long incubations.

All these features, and the fact that Petaka does not require a humid incubation environment, make it one of the most contamination protected cell culture devices available.



PetakaG3™

cell transport

With the Petaka you can transport live cells for long distances at normal temperatures. Petaka does not need dry ice or refrigeration to transport cells.

Its slim shape permits easy packaging and protection. Padded envelopes are ideal for shipping one or several units.

Cells inside Petaka, at normal temperatures, remain in a dormant state for days, even weeks. Viability decreases progressively depending on the cell type. CHO-K1 cells, for example, can travel for 3 to 4 weeks maintaining 75% viability. Some tumor cells can travel for a month or more.

PetakaG3™

cell dormancy

In Vitro cell dormancy

One main objective of Petaka technology is to provide freedom to researchers by reducing demanding time consuming routines.

Using regular flasks, cells should be subcultured as soon as possible when they reach a confluent monolayer state or saturate the carriers.

With Petaka, grown cell cultures can be maintained alive at room temperature, without dehydration risk, for long time periods.

This facilitates cell culture management and maintenance routines; and provides additional advantages for cell biology and toxicology research (see "Petaka Quick Protocols" for conditions and methods, such as protocols 4 and 8 - "Keeping Cells in In-Vitro Dormancy").

Cell conservation in dormant state is favored by transferring cultures from 37° C to 22° C when cells are in a slow progressing cell cycle. This is achieved in an 80% confluent state.

BASICS

Oxygen is the basic element for the cell's aerobic respiration, which is a highly efficient energy source. In its absence, energy is generated in the nucleated cell by anaerobic respiration, which is only 5% as efficient as aerobic.

Therefore, cultured cells need oxygen, but not in excess. When cells are cultured in media in classic cell culture devices, such as flasks and petri dishes, excessive oxygen is provided. In these devices, the media surface forms an interface with the atmosphere, which contains about 20% oxygen; through that interface, the oxygen in the air dissolves in the media up to the limit of its solubility in water, depending on cell culture temperature, atmospheric pressure and the degree of salinity of the media. This results in oxygen concentrations that are too high for normal cells, which naturally live in the lower oxygen environment found in living bodies

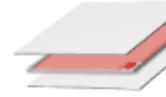
First

Withdraw Petakas from the incubator when cells become around 75% confluent.



Second

Protect both sides of Petaka with pieces of light cardboard.



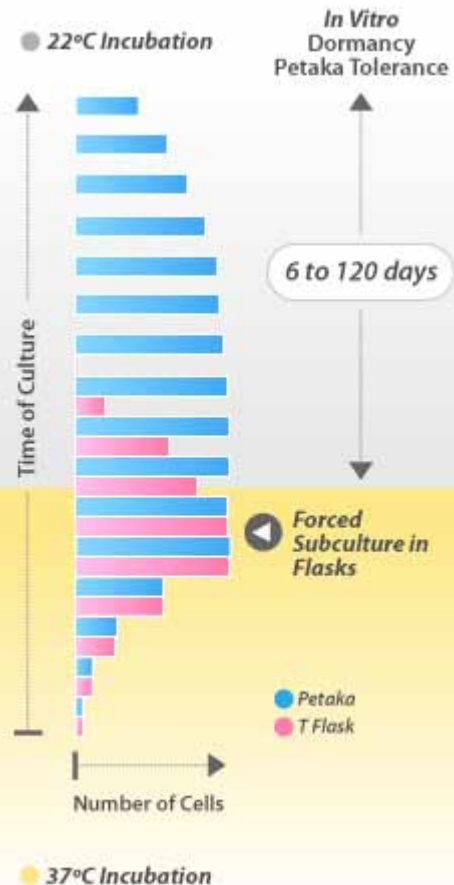
Third

Place the Petaka in a padded envelope



Fourth

Ensure that during transit Petaka will not be exposed to temperatures below 10°C or above 39°C (optimal temperature 20–22°C).
Optimal traveling time is 7 to 10 days



PetakaG3™

safe handling

1

Petaka's auto-sealing port, a pre-slit, 1 mm thick, silicon diaphragm, allows easy penetration with plastic and metal tips up to 1 mm external diameter.

2

Petaka's 0.2 mm Filtered Vent: The internal chamber communicates with the external atmosphere through an in/out vent closed with a 0.2 mm pore filter.

3

By avoiding caps and lids, the duo vent-port simplifies the repetitive operations of cell culture and handling, and minimizes routine operations.

BASICS

When the user initiates the injection procedure, the fluids flow through the tip into an atrium communicating with the culture chamber through a protective mini-tube. Therefore, during injection, the interior of the cell culture chamber is never exposed to the external atmosphere, through any aperture. In addition the mini-tube breaks any back pressure, avoiding leaks through the port slit.

The diaphragm surface is accessible for swabbing with ethanol, and can be exposed to a gas flame in order to guarantee surface sterility.

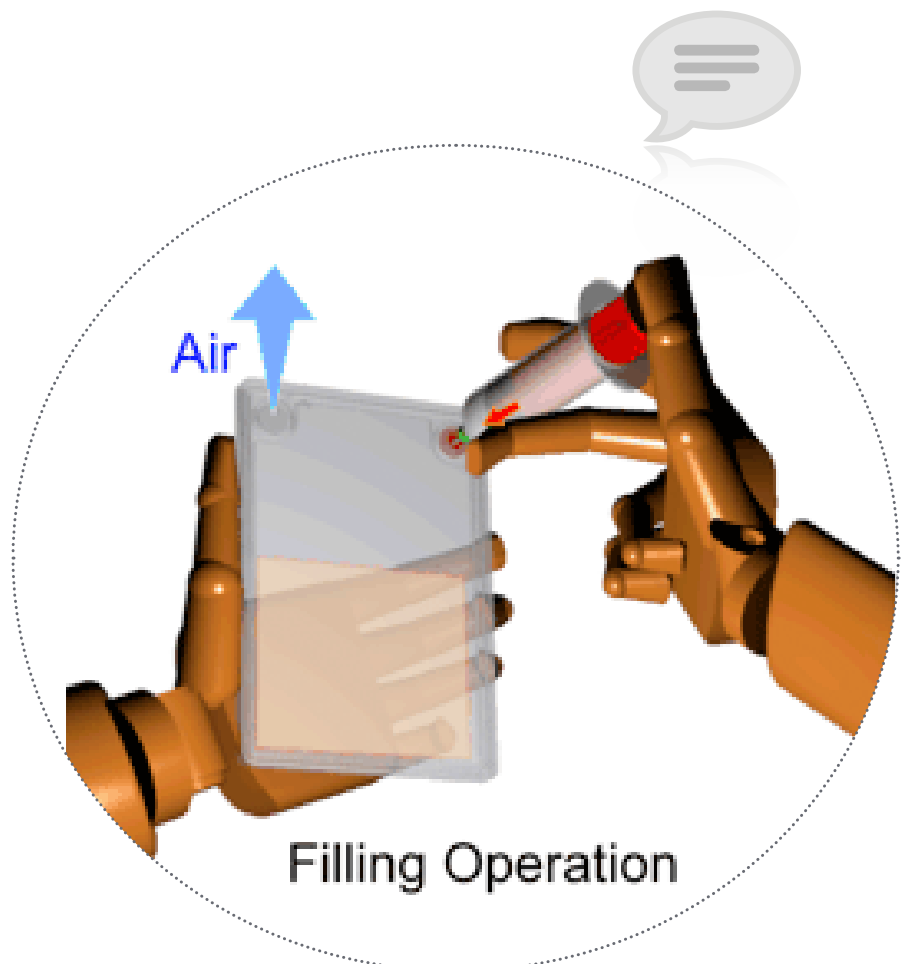
The port assembly allows safe and quick in/out maneuvers with reliable seal, greatly minimizing contamination risk.

The filter avoids open atmosphere exposure of the internal chamber. Air entering or exiting the chamber is therefore filtered, avoiding microbial contamination from the atmosphere during liquid handling operations.

Both port and filter permit easy fluid introduction and withdrawal, without exposing the interior chamber to the open atmosphere. When medium is introduced, the air excess is automatically and simultaneously expelled. Vice-versa, during withdrawal operations, air flows automatically through the 0.2mm filter into the internal

chamber, equalizing internal pressure with atmospheric pressure.

The proper use of Petaka ensures that the internal chamber will only contain filtered sterilized air. Additionally, air that flows from the internal chamber to the environment is also filtered, avoiding possible cross-contamination and accidentally contaminated cultures.



PetakaG3™

Basic Specifications

General Characteristics

Disposable device	Single use
Footprint	Microtiter plate
Color	Clear
Cell growth surface area	Nominal 150 cm ²
Volume of Media	20 ml.
Air space	< 0.5 ml.
Total space occupied per unit	53 cm ³
Total weight without media	32,000 mg. .
Total weight with media	57,000 mg.
Average density without media	1.05
Material	Crystal Polystyrene

Cell 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	1 mm
Growth surface color	Clear
Space between growth surfaces	3 mm +/- 0.2 mm
External surface tension (energy)	32-38 dynes/cm
Internal surface tension (energy) (Corona treated)	50-54 dynes/cm

Vent properties

Filter pore size	0.2 microns
Filter Characteristics	Hydrophobic
Filter material	PTFE : Polytetrafluoro Ethylene combined with 100% polypropylene support components. All materials conform to FDA requirements. 100% integrity tested and lot coded.

Water loss (Dehydration)

Spontaneous dehydration	
38°C and 90% Humidity	0.015 to 0.006 ml./day
38°C and 10% Humidity	0.140 ± 0.01 ml./day
22°C and 50% Humidity	0.001 ± 0.0001 ml./day

Access Port

Number Access Ports	1
Shape	Circular
Color	Orange
Size	OD 6 mm.
Port material	Biocompatible, Non-cytotoxic, Elastomer Exempt of: free radicals, surfactants, detergents, organic solvents, pyrogenic components
Surface Disinfectable with I/O Access device	Alcohol, Benzalkonium chloride, etc.
Atrium volume	16-20 gage blunt canulae/tips (ID up to 1000 mm.)
Buffer reservoir volume	< 50 ml.
Port operation	80-100 ml. Pressure penetration, auto-sealed

Optical properties

Refraction Index	1.57
Light Absorption Spectrum	From 310 nm to 670 nm absorbance < 1%
Autofluorescence w/	< 485 nm
Admitted optical resolution	0.25 mm.

Petaka Sterilization

Factory packaged	Gamma sterilized
After first use	Not autoclavable
Sterilization tolerance	Admits ETO, UV, and Gamma radiation

PetakaG3™

the cell culture new era

"The goal of cell culture engineers is no longer to simply increase a product's yield; it is to increase the product's yield without changing any key product characteristics. Excellent analytics has to be coupled with excellent cell culture to support the **Comparability Protocol** that will allow the process changes to be approved"
The Scientist, 2002.

"Companies are refining and streamlining the tools scientists need to accomplish cell culture, with an eye toward developing supplies that are easy to use".
Kathryn S. Brown, The Scientist, 2001.

"Continuous refinements to cell culture techniques and instrumentation, can make cell culture more a science than an art". *Genetic Engineering News, June 2001.*

"...Evidently, cell culture labs need better technical solutions in order to handle more cell lines and analyze more proteins...". *Cell culture is now a bottleneck; Cort Wrotnoswski; Genetic Engineering News, November 2000.*

"...Better tools and better techniques are needed in order to overcome today's cell culture technology limitations".

the answer to today's Biotechnology demands

With this revolutionary system Cell Culture is a Science and a Routine that rest on only three basic pillars:

First | Cells
Second | Media
Third | PetakaG3™

“
Petaka is a truly revolutionary product, the centerpiece of the cell culture-as-a-science concept, poised to displace the cell culture-as- an-art definition
”



transforming the art of the cell culture
into a sciential engineering

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