Cell Culture Pausing or In vitro Cell Dormancy

Logistic Cell culture pausing... Medium term cell storage without freezing
Cell cycle arrest, an established instrument in cell culture technology. Combination of factors.

Industrial applications of cultured cells developed methods to increase the production of recombinant proteins in mammalian cell cultures by reducing in-culture growth through cell cycle progression suspension. This cell cycle suspension increases the proportion of cells in the G1-phase, which subsequently increases their specific protein productivity (1). The induction of this arrest can be induced through cytostatic agents such as Sodium butyrate (NaBu) (2), dimethyl sufoxide (3), over-expression of cell cycle inhibitory proteins p21Cip1 (4) or through control of the modification of the culture environment including simple mild hypothermia(5).

Mild hypothermia is a simple and innocuous procedure but whilst it is an easy application in suspended cell cultures maintained in tank bioreactors there are several difficulties to be applied in small culture flasks and dishes where dehydration is a limiting factor. Incubation at 28 °C or 30 °C, necessary for inducing cell cycle arrest, is not high enough to obtain the level of environmental relative humidity required for blocking the media evaporation.

Petaka proprietary controlled diffusion system prevents dehydration for weeks and months at any temperature maintained in 10% relative humidity environment. Therefore, cell can be incubated in Petaka at 20 °C or 30 °C with no risk of accidental dehydration, achieving al level of hypothermia compatible with cell cycle reversible arrest.
A classical cellular response to **hypoxia** is a pause of growth (6). Hypoxia-induced cell growth arrest differs in diverse cell types but is likely an essential aspect of in vivo tissue repair control in the response to wounding and injury. A major constituent of the hypoxic response is the activation of the hypoxia-inducible factor 1 (HIF-1) transcription factor through the involvement of the tumor suppressor protein p53 (7).

Hypoxia (0.5% O₂ = 1.3 mmHg) can alter cell proliferation in two distinct ways: via programmed cell death and through growth arrest. In transformed cells, hypoxia can provoke apoptosis via the p53 pathway and p27 expression (8). However, mild hypoxic environment can induce cell cycle arrest at the G1/S interface without any alteration in their long-term viability.

**Acidosis** also has a major role in controlling the cells growth through the key functions of p53 (9). Below pH 7 glucose consumption (10) is reduced in cultured cells and cell cycle become slower up to 0.2 times the cell cycle time at pH 7.4. Consequently a precise combination of all these three factors induce a reversible arrest of the in vitro cell proliferation, maintaining the cells in a kind of cell growth and metabolic pause, or in vitro cell culture dormancy.
Cell cycle arrest, an established instrument in cell culture technology. REFERENCES


Unique Petaka Application – In vitro Cell Dormancy

It is a totally unique option to be able to perform this phenomenon in vitro in Petaka.

No complex protocol is required. When a culture is around 75% confluent, simply remove the Petakas from the heat source (normally an incubator) & maintain in an ambient temperature of around 22 °C (18 to 24°C) in a dark space.

The 4 primary factors effecting this condition are:

1. Mild hypothermia (room temperature 22 °C)
2. pH of the media which is at its optimum for dormancy when at 90% confluent (pH of around 6.8)
3. Mild hypoxia which is at its optimum for dormancy when at 90% confluent (2 ppm of dissolved oxygen)
4. Isolation from light source (avoiding any IR source of energy)
Unique Petaka Application – In vitro Cell Dormancy

There are 3 principal unique applications possible using Petaka using the in vitro dormancy which is literally impossible to replicate in any other device

1. In vitro shipping of cells – Avoiding the necessity of freezing or dry ice, large quantities of cells can be shipped international at a fraction of the price reducing logistics cost up to 80%. On arrival, cells would have avoided the double handling & exposure to stress causing freezing media & enabling the cell culture to be examined at the researchers own convenience.

2. Cell culture pause – Enabling easy nonsense-free switching from one experiment to another. As priorities change (awaiting suitable media or growth factor), a culture may be put on hold for hours, days or even weeks. May be considered as a short to medium term cell storage option.

3. The unique dormancy option may well open the doors to researchers for investigating parallels with cancer cell dormancy in humans.
So what is in vitro cell dormancy?

Is a normal consequence of these three coincident events:

- Hypothermia 22°C
- Acidosis pH 6.9
- Hypoxia 2 ppM DO
Petaka has a special virtually hermetic closure which provides extraordinarily reduced levels of water vapor leaks; protecting cultures in Petaka against dehydration in dry environments.

This allows the cell cultures to be kept alive in nearly any environment, even at relatively low temperatures, which together with physiologic oxygen concentration preserves cell life over four times longer than in traditional flasks.
The state of the cells is like a suspended animation, with slowed cell cycles, or cells dormancy, which depending on cell type, may be prolonged to over 35 days.
Biological conditions uniquely achievable in Petaka

1. Cells enter a completely reversible dormant state by maintaining cultures in Petaka under a mild hypothermic environment (18-24°C) for up to several weeks at a time.
2. During dormancy, the cell cycle is arrested in G2 phase.
3. Returning to the regular cell cycle again only requires returning the culture to euthermia (37°C).

No effects to the genomic structure

“In vitro suspended cell animation or…”

“In vitro Cell Dormancy”
Unique Petaka Application – In vitro Cell Dormancy

So what is in vitro cell dormancy?

It is an environmental cell adaptation characterized by... the cell cycle slowing down, with cells in G1 & Mitosis passing through slowly to be arrested and gathered in the G2 phase.................
So what is in vitro dormancy?

It is an environmental cell adaptation characterized by... the cell morphology going from being stretched out (spindle shaped) or flat (polygonal) to a more rounded mushroom shape with a much smaller area of contact to the bioreactor surface....
Unique Petaka Application – In vitro Cell Dormancy

So what is in vitro dormancy?

It is an environmental cell adaptation characterized by...........the cells experiencing a significant drop in their oxidative metabolic activity...............
It is an environmental cell adaptation characterized by the slowed cell cycle (or arrested), significant reduction in consumption of oxygen & nutrients. The cells may remain in this state for weeks or months, depending on cell type. This allows the cells to be shipped in purpose made insulated bubble mailers in a package a fraction of the size of those usually frozen for deliveries with dry ice.

This condition is entirely reversible, leaving no change to the genomic integrity of the cell.
Petaka

Unique Petaka Application – Laboratory organization independent of the cell cultures times

It is an environmental cell adaptation characterized by the slowed cell cycle (or arrested), significant reduction in consumption of oxygen & nutrients, the cells may remain in this state for weeks or months, depending on cell type. This allows the cell cultures to be paused at any moment, based on laboratory strategy not dependent of the cell culture servitude.

This condition is entirely reversible, leaving no change to the genomic integrity of the cell.
**Petaka**

1. Hypoxia experimentation in Petaka
2. Long term cell storage freezing at -196°C in Petaka
3.Repeated sampling of the cell culture throughout its incubation for PAT
4. Logistic Cell culture pausing... Medium term cell storage without freezing
5. Magnetic cell sorting and transfection
6. In Petaka cell centrifugation, pelleting and washing
7. Automatic Enzyme free cell harvesting
8. Tissue like environment
9. Cell Shipping without freezing
10. Time lapse video on using any microscope without special chambers.

**Celartia**

**Petaka the Cell Management Device**