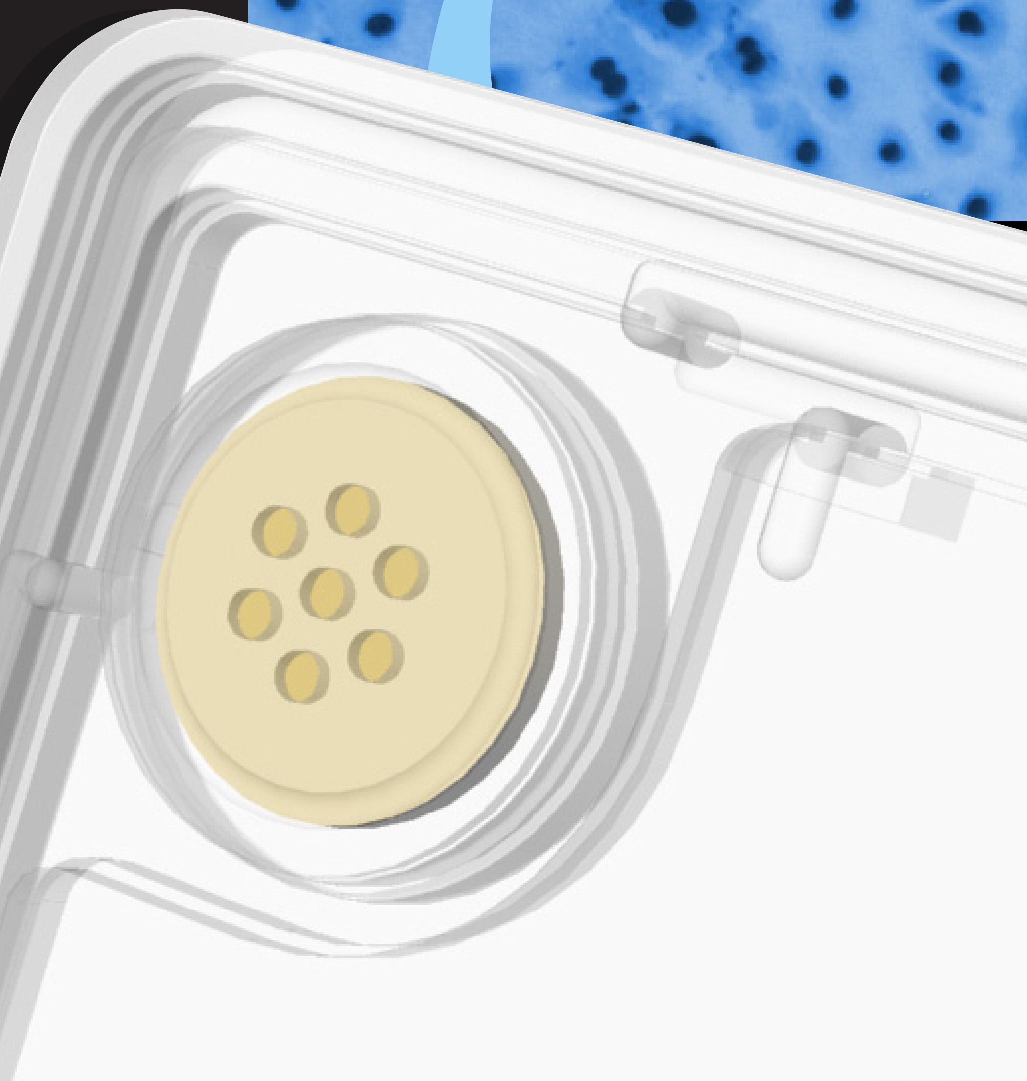
Life Technologies (India) Pvt. Ltd.
your molecular & cell technology partner

India Contact:
Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura,
Delhi – 110034 (INDIA).
Ph: +91-11-42208000, 42208111, 42208222
Mobile: +91-9810521400
Fax: +91-11-42208444
Email: customerservice@lifetechindia.com
Web: www.lifetechindia.com

PetakaG3™

Cell Culture Common Quick Protocols

www.petaka.com
life in it!



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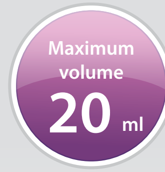
Basics

- Maximum volume of media permitted in Petaka is



(more will alter Petaka's performance!)

- Media injection and withdrawal should always be done holding Petaka in a VERTICAL POSITION

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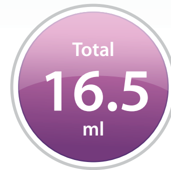
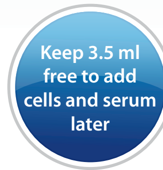
Ready to host Petakas (RTHP)

PREPARING "READY TO HOST" PETAKAS

- Hold the Petaka in a vertical position!
  1. Inject 0.5 ml of Antibiotic solution
  2. Pump 16 ml of Medium





  3. Keep these Petakas refrigerated at 4ºC in a vertical position in stands or in a plastic bag (zip bag)
- Dehydration and pH should be controlled if RTHP are kept In refrigerator for months!

SEEDING CELLS IN "READY TO HOST" PETAKAS

- Holding Petaka a in vertical position

1. Disinfect port with 90% Ethanol & a flame
2. Warm the Petakas in an incubator, at 37ºC for 20 -30 min in a VERTICAL position
3. Inject 1.5ml of serum (*)
4. Inject up to 2ml of cell suspension
5. Whilst holding the Petaka in a vertical position, squeeze it until the level of media reaches the air exit & then release until the air input stops. Then the inside/outside pressure will be balanced
6. Shake gently for 10-15 seconds
7. Place in a stand & allow to incubate in a HORIZONTAL position

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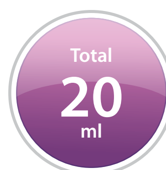
Culturing cells in media supplemented media

SEEDING THE CELLS

- Holding Petaka in a vertical position!

1. Inject 1 ml of Serum for 5% concentration
2. Inject 0.5 ml of Antibiotic solution
3. Inject X ml of Cell suspension
4. Inject (18.5 –X) ml of Medium



5. Whilst holding the Petaka in a vertical position, squeeze it until the level of media reaches the air exit & then release until the air input stops. Then the inside/outside pressure will be balanced.
6. Incubate Petaka according to the Cell type (see INCUBATION POSITIONS)

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Harvesting anchored cells

ADHERENT CELLS WITH TRYPSIN-EDTA

1. Disinfect the port with 90% Ethanol & a flame
2. Completely extract all the media from the Petaka
3. Inject 4 ml of 0.25% Tyrpsine-EDTA solution
4. Shake thoroughly
5. Incubate at 37ºC for 3 min in a horizontal position (both sides)
6. Check Cell detachment under the microscope
7. WHEN CELLS ARE DETACHED! Tap the Petaka 2-3 times
8. Pellet the cells using a Petaka centrifuge (see centrifugation)
9. To pellet the cells in the bottom right hand corner of Petaka, position it with the port being the upper most corner of the device
10. Introduce Petaka into the centrifuge rotor pocket
11. Set desired speed & time & begin centrifuge
12. After centrifuge, the cell pellet will be visible in the bottom right hand corner
13. To extract the pellet, use a micro pipette with a 1mm tip
14. Transfer the cells to a conical tube

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Harvesting free suspended cells

NON ADHERENT CELLS

1. Disinfect port with 90% Ethanol & a flame
2. Tap the Petaka 2-3 times
3. Pellet the cells using a Petaka centrifuge (see centrifugation)
5. To pellet the cells in the bottom right hand corner of Petaka, position it with the port being the upper most corner of the device
6. Introduce Petaka into the centrifuge rotor pocket
7. Set desired speed & time & begin centrifuge
8. After centrifuge, the cell pellet will be visible in the bottom right hand corner
9. To extract the pellet, use a micro pipette with a 1mm tip
10. Transfer the cells to a conical tube

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Keeping cells in “in vitro dormancy” (only adherent cells)

PUTING THE CELLS IN “IN VITRO DORMANCY”

1. After 75% confluent cell growth, remove the Petakas from the incubator
2. Keep the Petakas at 20-25ºC in a vertical position, away from direct sunlight, or Infra-Red sources.
3. Keep in a Polyspan Shield
4. Periodically Check Cell shape under the microscope

DO NOT CHANGE THE MEDIUM!

In-dormancy survival time depends on:
a) Cell type
b) Culture Media
c) Type of Buffer (in the media)
d) Stability of the environmental variables

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Recovering cells from “dormancy”

RECOVERING CELLS FROM “DORMANCY”

1. Disinfect port with 90% Ethanol & a flame
2. Slowly withdraw the medium
3. Inject the same volume of NEW medium with the same additives
4. Whilst holding the Petaka in a vertical position, squeeze it until the level of media reaches the air exit & then release until the air input stops. Then the inside/outside pressure will be balanced.
5. Incubate at 37°C, in a vertical position, at least for 24 hours before cell SUBCULTURE

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pH adjustment of growing cultures

pH ADJUSTMENT WITH SODIUM BICARBONATE

1. Disinfect port with 90% Ethanol & a flame
2. Withdraw 1ml of Medium
3. Whilst holding the Petaka in a vertical position, squeeze it until the level of media reaches the air exit & then release until the air input stops Then the inside/outside pressure will be balanced
4. Inject 1ml of 7.5% Sodium Bicarbonate sterile solution
5. Shake gently for 10-15 seconds.
6. Return the Petakas to the incubator

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Media change

MEDIA CHANGE

1. Disinfect port with 90% Ethanol & a flame
2. Slowly withdraw all the medium completely
3. Inject the same volume of NEW medium with the same additives
4. Whilst holding the Petaka in a vertical position, squeeze it until the level of media reaches the air exit & then release until the air input stops. Then the inside/outside pressure will be balanced.
5. Incubate at 37°C, in a vertical position, at least for 24 hours before cell SUBCULTURE
6. Return Petaka to the incubator

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Freezing cells

- First follow the harvesting cells protocol up to the pellet formation and extraction.

1. Disperse the cell pellet in FREEZING MEDIUM at a final concentration: 1,000,000 cells per ml of medium
2. Transfer 1.5 ml of cell suspension into each Cryotube.
3. Proceed freezing the Cryotubes (stepwise) up to -120ºC & store in LIQUID NITROGEN

- In order to remove the media whilst keeping the cells within the Petaka we need to gather the cells in the bottom left hand corner, therefore for centrifuging the air filter must be in the uppermost corner of the Petaka

4. Place the Petaka in the centrifuge, set speed & time, then begin
5. Remove Petaka from the centrifuge
6. Pellet cells will have gathered in the bottom left hand corner
7. Whilst holding the Petaka upright with the pellet cells in the bottom corner, slowly remove the media, careful not to disturb the pellet cells
8. Repeat centrifuge 2 more times ensuring the port is disinfected each time

- Then to collect washed pellet cells, inject new medium, place the Petaka in the centrifuge rotor pocket with the port being in the upper most corner.

9. Set the centrifuge speed & time & begin
10. Remove the Petaka from the centrifuge
11. Note the pellet cells in the bottom corner under the port
12. Remove the pellet cells using a micro pipette
13. Transfer into a conical tube containing freezing media
14. Transfer this into a cryotube
15. Place in the freezer at least -80ºC or in liquid nitrogen

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Thawing frozen cells

1. PREPARE “READY TO HOST” PETAKA WITH ONLY 15ml OF MEDIA
2. Disinfect the port with 90% Ethanol & a flame
3. Warm the RTHP in an incubator, at 37ºC for 10-20 minutes in a VERTICAL position.
4. Inject 3ml of Serum (*)
5. Remove the cells from the freezer
6. Thaw the cryotube in a disinfected water bath at 38ºC for 1-3 minutes.
7. Be sure the ice has melted
8. Extract the cell suspension from the cryotube with a 1ml tip.
9. Transfer the cell suspension into a Petaka(RTHP)
10. Whilst holding the Petakain a vertical position, squeeze it until the level of media reaches the air exit & then release until the air input stops. Then the inside/outside pressure will be balanced.
11. Shake gently for 10-15 seconds
12. Incubate the Petaka in a horizontal position according to the Cell type (see INCUBATION POSITIONS)

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In Situ monitoring adherent cells viability

TRYPAN BLUE METHOD

1. Disinfect port with 90% Ethanol & a flame
2. Withdraw 1ml of Medium
3. Inject 1ml of Trypan Blue 0.4% “sterile” solution
4. Shake gently for 5-10 seconds
5. Leave the Petaka in a vertical position for 10 minutes at room temperature
6. Count proportion of “blue-stained” cells (dead cells) versus unstained per field (living cells)
7. Disinfect port with 90% Ethanol & a flame
8. Slowly withdraw the medium
9. Inject the same volume of NEW medium
10. Incubate in the required position

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Shipping cells in Petaka

PREPARING ADHERENT CELLS FOR SHIPMENT

1. Withdraw the Petakas from the incubator when cells become around 75% confluent
2. Protect Petaka with Polyspan Shield
3. Introduce Petaka/s in a mailer envelope
4. Ensure that during the trip Petaka will not be exposed to temperatures below 10ºC or over 39ºC (optimal temperature 20-22ºC)
5. Optimal traveling time is up to 7-10 days.

Intercontinental delivery times have been successfully up to 14 to 21 days; however, depending on the cell type, final viability at destination could be as low as 30-50%

RECOVERING CELLS AFTER SHIPMENT

1. Disinfect port with 90% Ethanol & a flame
2. Slowly withdraw the medium
3. Inject the same volume of NEW medium
4. Whilst holding the Petaka in a vertical position, squeeze it until the level of media reaches the air exit & then release until the air input stops. Then the inside/outside pressure will be balanced.
5. Incubate at 37ºC, in vertical position, at least for 6-12 hours before cell SUBCULTURE

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Tissue cell isolation and primary culture

1. Inside the laminar flow hood or in the operating room, excise a tissue sample and place into a 10 cm tissue culture dish containing approximately 10 ml of Hanks Balanced Salt Solution (HBSS).
2. Inside the laminar flow hood, remove any fat and necrotic areas from tissue sample. Maintain a sterile environment all times.
3. In a minimal amount of HBSS, thoroughly mince the tissue sample into a fine slurry of 1mm3 or smaller particles
4. Transfer the minced tissue sample into a 50 ml conical tube and spin down at 2000 rpm for 3 minutes in a refrigerated centrifuge
5. Fill the 50 ml conical containing the collagenase digest up to 40 ml with HBSS and Spin at 3000 rpm for 5 minutes. Discard the supernatant.
6. Add warm DMEM with 10% FBS and 1% antibiotic, up to 10 Ml and disperse the cell pellet.
7. Suck up the mixture into a 10ml plastic pipette and eject it, repeating both operations 10X in order to release free cells.
8. Pass the mixture through a 100µm filter (cell strainer) into a new 50 ml conical tube.
9. Count cell concentration and seed two million cells in 1-5 ml suspension per Petaka.
10. Add media with 10% FBS and 2% Penicillin/Streptomycin solution up to 20 ml per Petaka.
11. Incubate in the adequate position

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