

## **PROTOCOL**

# **“Anchored Cell detachment, concentration and cell wash for further studies”**

Without Celartia Centrifuge

## PETAKA G3 DESCRIPTION

|  |  |
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|  | <b>Petaka G3</b>   |
| <b>Biotechnology</b>                     | <b>New. Patented</b><br><br><b>Self regulated gas diffusion system.</b>  |
| <b>Air space (volume lost)</b>           | <b>3.8 % of the total volume</b>   |
| <b>Surface available for cell growth</b> | <b>150 cm<sup>2</sup></b>  |
| <b>Average Cell Production</b>           | <b>20 million cells</b>  |
| <b>Device volume</b>                     | <b>59 cm<sup>3</sup></b>   |
| <b>Design</b>                            | <b>New. Patented.</b><br><br><ol style="list-style-type: none"><li><b>1. Adapted to robotic cell culture</b></li><li><b>2. Prepared for internal cell concentration by centrifugation.</b></li><li><b>3. Prepared to segregate cells from supernatant.</b></li><li><b>4. Prepared for direct positive and negative immune-magnetic cell sorting</b></li><li><b>5. Prepared for Microgravity cell culture (orbital incubation)</b></li><li><b>6. Prepared for long distance cell shipping without freezing or cooling.</b></li><li><b>7. Prepared for induction of “in vitro cell dormancy”</b></li></ol> |

## PROTOCOL.

### Adherent Cell Transfer without Petaka Centrifuge

#### Materials needed

PetakaG3  
Flame  
30 mL Syringe  
Petaka tips  
10 mL conical tubes  
Laminar flow hood  
Centrifuge  
Cell counter

### 27% savings in instrumentation

Disinfect Petaka port with 90 % Ethanol and flame it briefly

Time:0.1 min



Completely withdraw the media

Time:1 min



Inject 4 mL of Trypsin solution

Time:0.1 min



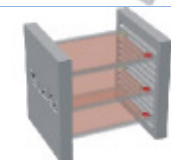
Rotate and gently shake the PETAKA to cover all cell culture surface with a thin layer of trypsin

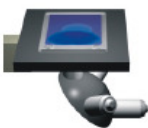



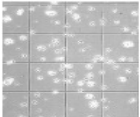


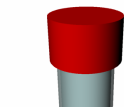



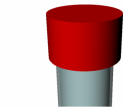
Time: 1 min






Incubate for 3 to 4 minutes at 37°C

Time 4 min



|  |   |
|--|---|
| <p>Check cell detachment under the microscope<br/> <b>Time: 1 min</b></p>  |    |
| <p>Disinfect Petaka port with 90% Ethanol and flame it briefly<br/> <b>Time:0.1 min</b></p>  |    |
| <p>Inject into Petaka 6 mL of media having 20% FBS to inhibit the trypsin activity<br/> <b>Time: 0.1 min</b></p>   |    |
| <p>When the injection is complete, withdraw a small sample of cell suspension<br/> <b>Time: 0.5min</b></p>   |    |
| <p>Count the cell content in cells/mL and estimate the total amount of cells during the first centrifugation<br/> <b>Time:5 min compensated by centrifugation time</b></p> |    |
| <p>Completely withdraw the media<br/> <b>Time:1 min</b></p>  |   |
| <p><b>IN LAMINAR FLOW HOOD.</b><br/> Transfer cell suspension to a 10 mL conical tube Close the tube with cap.<br/> <b>Time: 1 min</b></p>                                 |  |
| <p><b>Close the tube with the cap</b></p>  |  |
| <p>Centrifuge the conical tube at the necessary RPM and for sufficient time to obtain a soft cell pellet<br/> <b>Time: 6 mins</b></p>                                      |  |
| <p><b>IN LAMINAR FLOW HOOD.</b><br/> Decant the media keeping intact the cell pellet.<br/> <b>Time: 0.1 min</b></p>  |  |
| <p><b>IN LAMINAR FLOW HOOD.</b><br/> Add 10 mL of PBS to the conical tube and vortex it until complete cell suspension.<br/> <b>Time: 2 mins</b></p>                       |  |
| <p><b>Close the tube with the cap</b></p>  |  |

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|---|---|
| <p>Centrifuge the conical tube at the necessary RPM and time to obtain a soft cell pellet<br/><b>Time: 6 mins</b></p>                         |  |
| <p><b>IN LAMINAR FLOW HOOD.</b><br/>Decant the media keeping the cell pellet intact .<br/><b>Time: 0.1 min</b></p>                            |  |
| <p>Transfer the cells to the desired device adjusting volumes<br/><b>TOTAL Time: 24.1mins    15.7% savings time compared to T75 flask</b></p> |  |