PriCells: Subculture of Culture Primary Cells

Note: PriCells primary cells for research use only.

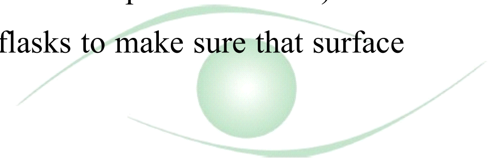
The number of subcultures (passages) that can be achieved will vary with either the starting primary cell density and activity or the methods employed by individual investigators and researcher. View the culture under a microscope to make certain the condition of the primary cell culture (i.e., density, confluence, mitotic activity). The procedure given below is a sample protocol for the subculture of the T-flasks. If different-sized culture vessels are to be used, reagent volumes should be adjusted accordingly.

Note: Do not warm the reagents before using. We recommend using culture surfaces that have been coated with PriCells factor for cell culture attachment (www.pricells.com or www.pricells.com.cn) or PriCells flasks (www.pricells.com or www.pricells.com.cn)

1. Prepare new culture T-flasks coated with PriCells factor for cell culture attachment (www.pricells.com or www.pricells.com.cn) or from PriCells (www.pricells.com or www.pricells.com.cn).

2. Remove all of primary cell culture medium from T-flasks.

3. Add 3 ml of Trypsin/EDTA solution (www.pricells.com or www.pricells.com.cn) to T-flasks upon investigators and researcher. Slightly rock T-flasks to make sure that surface of T-flasks is covered with Trypsin/EDTA solution.



Note: For an appropriate Trypsin/EDTA solution concentration, please refer to the appropriate document or contact www.pricells.com or www.pricells.com.cn.

4. Return culture of primary cells to a 5% CO_2/95% air, 37°C, and humidified incubator, approximately a few minutes upon researchers and investigators.

5. View the culture under a microscope. Incubate the cells at room temperature until the cells have become completely round, approximately a few minutes upon researchers and investigators.

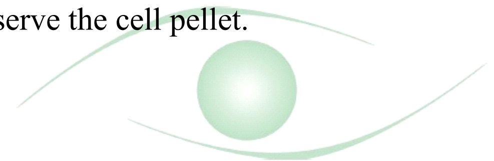
6. Rap the flask gently to dislodge the cells from the surface of T flasks.

Note: For an appropriate Trypsin/EDTA solution time in T flasks, please refer to the appropriate document or contact www.pricells.com.

7. Add 3 ml of Trypsin neutralizer solution (www.pricells.com or www.pricells.com.cn) to T flasks (www.pricells.com or www.pricells.com.cn) and transfer the detached cells to a sterile 15 ml tube.

8. Add 3ml additional Trypsin neutralizer solution (www.pricells.com or www.pricells.com.cn) to T flasks and pipet the solution over surface of T flasks several times to remove any remaining cells. Add this solution to the 15 ml tube.

9. Centrifuge primary cells at 180 × g for 5-7 minutes. Observe the cell pellet.



10. Remove the supernatant from the 15 ml tube.

Note: NOT to remove the cell pellet, carefully.

12. Re-suspend primary cell pellet in 4 ml primary cell culture system (www.pricells.com or www.pricells.com.cn). Pipet primary cells up and down with a 10 ml pipette to ensure a suspension of homogeneous cell pellet.

13. Remove 20 µl from the tube and dilute primary cell suspension in 20 µl trypan blue solution (www.pricells.com or www.pricells.com.cn).

14. Using a hemacytometer, determine the number of viable cells per ml.

15. Dilute the contents of primary cells to a concentration of 2.5×10^4 viable cells/ml using primary cell culture system (www.pricells.com or www.pricells.com.cn).

16. Incubate culture of primary cells in a 37ºC, 5% CO_2/95% air, and humidified cell culture incubator. For best results, do not disturb the culture for at least 24 hours after the culture has been initiated.

Note: To achieve the highest cell densities, change the primary cell culture system (www.pricells.com or www.pricells.com.cn) every day as the cultures approach confluence. In general, primary cell cultures seeded at 5.0×10^3 cells/cm^2 from cryopreserved cells should reach 80% confluency in 5-7 days upon primary cell medium and other factors.

17. For further instructions on maintenance and subculture of the cells, you can refer to the appropriate document or contact www.pricells.com or www.pricells.com.cn.

Caution

1. Trypsinization: Cultured primary cells can be harmful when exposure of the cells to the Trypsin/EDTA solution for excessive lengths of time can occur during trypsinization.

2. Centrifugation: Centrifugation of primary cells at excessive g forces is also harmful, especially, cryopreserved primary cells.

3. Although cryopreserved cells are tested for the presence of various hazardous agents, diagnostic tests are not necessarily 100% accurate.

4. Primary cells may haven other known or unknown agents, or organisms that could be harmful to your health or your environment.

5. You MUST wear protective clothing and eyewear during processing culture of primary cell. The appropriate disposal techniques for potentially pathogenic or biohazardous materials MUST be used in your procedures.

6. In case of contact with eyes, you MUST rinse immediately with plenty of water and seek medical advice.