PriCells

PriCells: Beginning Cultures from Cryopreserved Primary Cells

Note: PriCells primary cells for research use only.

Usually, four 25 cm^2 flasks can be established from one vial containing >5 × 10^5 primary cell. We recommend seeding primary cells recovered from cryopreservation at a density of 5.0 × 10^3 viable cells/cm^2 of culture area.

Note: 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) before initiating cultures from cryopreserved primary cells. The procedure given below is a sample protocol for establishing cultures from the contents of one vial of cryopreserved storage.

1. Prepare a bottle of primary cell culture system according to the instructions.

2. Remove one vial of primary cells from liquid nitrogen storage, taking care to protect hands and eyes (see below **Caution**).

3. Dip the lower half of the vial into a 37°C water bath to thaw.

4. When the contents of the vial have thawed, wipe the outside of the vial with 70% ethanol or isopropanol.

5. Open the vial and pipet the suspension up and down with a 1 ml of pipette to disperse the cells.

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

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

8. Dilute the contents of the vial (1 ml) to a concentration of 2.5×10^4 viable cells/ml using primary cell culture system.

9. Add 5 ml of primary cell system (www.pricells.com or www.pricells.com.cn) to each 25 cm^2 culture T flasks (www.pricells.com or www.pricells.com.cn) that have been 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).

10. Following inoculation, swirl the media in the flasks to distribute the cells. Primary cells attach to culture surfaces quickly, and if the medium is not distributed immediately following inoculation, the cells may grow in uneven patterns.

11. Incubate culture of primary cells in a 37°C, 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 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.

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12. 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.