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Multiple Layer Tissue Culture Flasks
Application Guide

Culture Setup

1. Stand flask upright and dispense the desired amount of medium into the flask by pouring or using a serological pipet. The recommended volume to use is 25-50mL per layer. Dispense the medium gently in order to avoid foaming.
2. Add desired concentration of cell suspension, maximum 10mL, directly into the medium using a 10mL pipet.
3. Holding the flask upright, with the sloping top-side of the flask facing towards you, tilt the flask **counter-clockwise** 45° to ensure the cell suspension reaches the mixing port in the left corner.
4. Keeping the same 45° angle, gently rotate the multi-flask neck-side toward and away from you to mix the cell suspension.
5. After mixing, stand the flask upright again to allow the solution to equilibrate.
6. Holding the flask upright, with the sloping top-side of the flask facing towards you again, tilt the flask **clockwise** 45° to ensure the cell suspension equally distributes to all layers.
7. Lay the flask on a flat work surface and gently rock back and forth to ensure the cell suspension distributes evenly across the surface of all layers.

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Changing Medium

1. Aspiration Method:
    a. Stand flask upright and remove vent cap. Tilt the sloping top-side of the flask towards you, then tilt flask **counter-clockwise** at a 45° angle and aspirate medium directly from the flask.
    b. Tilt flask **clockwise** at a 45° angle to continue to aspirate residual medium.
2. Pour Method:
    a. Stand flask upright and remove vent cap.
    b. Tilt flask so that the sloping top-side is facing down. Pour medium directly out of the flask into an appropriate receptacle.
3. Add fresh medium by either pipetting or pouring (see step 1 of Culture Setup)

Harvesting Cells

1. Remove medium from the flask as outlined above (Changing Medium).
2. For difficult to harvest cells, first wash the monolayers with warm PBS, ensuring distribution to all layers (>5 mL per layer). Remove and discard PBS. This wash step may be repeated.
3. Add warm dissociating reagent (>5mL per layer) and ensure distribution to all layers. Apply gentle rocking, tapping, and/or brief (1-5min) incubation to maximize cell detachment as appropriate.
4. Neutralize the dissociating reagent:
    a. Add an equal or greater volume of fresh, full medium to the flask. Follow the steps outlined above to ensure full distribution to all layers. Collect the solution and add to the first collection of cells.
    b. If a significant amount of cells remain uncollected in the flask after step (a), then a second round of dissociation

reagent addition and collection is recommended before neutralizing.

c. If neutralization is not possible (i.e. serum-free conditions are required), centrifuge the collected cells, discard the medium, and resuspend the pellet in fresh medium.

5. Collect cells using the Aspiration Method or the Pour Method.