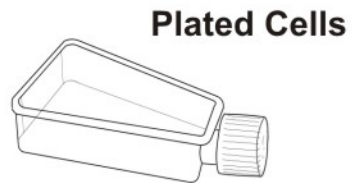


# Seeding Protocol

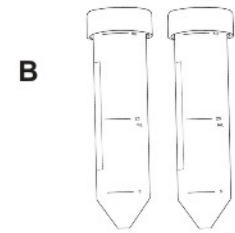


1. Wipe down flask with 70% Alcohol upon arrival.
2. Transfer in to Tissue Culture Hood

## Inside Tissue Culture Hood



3. Remove 100% Media and transfer into (2) 50ml Sterile Conical Tubes.



## Attached Cells in Flask



- A1. Wash with 2ml 1XPBS 2-3min remove and discard 100% 1XPBS Cat# P1408-013
- A2. Incubate at 37°C, 5% CO<sub>2</sub> for 2 Hrs. with 7ml of Complete Growth Media.

- A3. Remove and discard 100% Media
- A4. Wash with 2ml 1XPBS 2-3min Remove and discard 100% 1X PBS
- A5. Trypsinize Cells with 2ml of Trypsin EDTA Cat# T1509-014

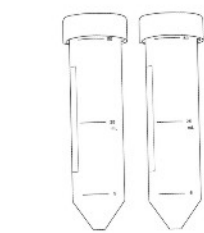
- A6. Neutralize Trypsin with 5ml of Fresh Complete Growth Media with Serum to bring total Volume to approx. 7ml.
- A7. Transfer into (1) 15ml Sterile Conical tube.
- A8. Centrifuge 15ml Conical Tube at 100g for 7min. to obtain Cell Pellet.



## Cell Pellet

- A9. Discard 100% Supernatant
- A10. Reconstitute Cells in 7ml of Complete Growth Media

- A11. Transfer 7mls of the Reconstituted Cells in a Pre-coated T25 Flask
- A12. Incubate at 37°C, 5% CO<sub>2</sub> in Humidified incubator.

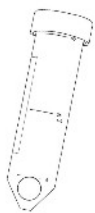


## Suspension Cells

- B1. Centrifuge the (2) Conical Tubes with Cell Suspensions at 100g for 7min.

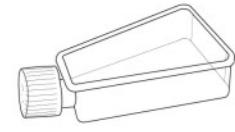
## Cell Pellet

- B2. Discard 100% Supernatant
- B3. Reconstitute Cells into 3.5ml of Complete growth Media per 50ml Conical Tube
- B4. Combine all Cell Pellets in the 50ml tubes into (1) 15ml Conical tube to reach a total volume of approx. 7ml.



## Plate Cells

- B4. Transfer 7mls of Reconstituted Cells in a Pre-coated T25 Flask
- B5. Incubate at 37°C, 5% CO<sub>2</sub> in Humidified incubator.



Inspect Cells after 24hrs of plating.

**Note: If the Suspension Cell density is low combine with attached Cell Pellet into a total volume of approx. 7ml per T25.**

**Note: If the attached Cells density is low, combine with Suspension Cell Pellet into a total volume of approx. 7ml per T25 Flask.**

**\*\*If Cell density is high, plate Cell Pellet into (2) T25 Flasks for 1:2 split.**