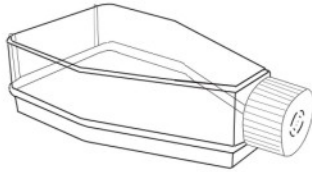


Plated Cells

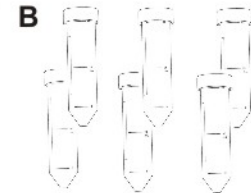


1. Wipe down flask with 70% Alcohol upon arrival.
2. Transfer in to Tissue Culture Hood

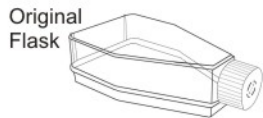
Inside Tissue Culture Hood



3. Remove 100% Media and transfer into (6) 50ml Sterile Conical Tubes.



Attached Cells in Flask



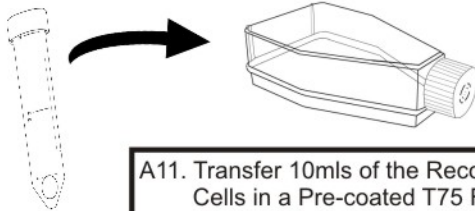
- A1. Wash with 3ml 1XPBS 2-3min remove and discard 100% 1XPBS Cat# P1408-013
- A2. Incubate at 37°C, 5% CO₂ for 2 Hrs. with 10ml of Media.

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

- A6. Neutralize Trypsin with 7ml of Fresh Complete Growth Media with Serum to bring total Volume to approx. 10ml.
- A7. Transfer into (1) 15ml Sterile Conical tube.
- A8. Centrifuge 15ml Conical Tube at 100g for 7min. to obtain Cell Pellet.

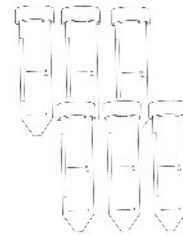
- A9. Discard 100% Supernatant
- A10. Reconstitute Cells in 10ml of Complete Growth Media

Cell Pellet



- A11. Transfer 10mls of the Reconstituted Cells in a Pre-coated T75 Flask
- A12. Incubate at 37°C, 5% CO₂ in Humidified incubator.

Suspension Cells



50ml Conical Tubes

- B1. Centrifuge the (6) Conical Tubes with Cell Suspensions at 100g for 7min.

Cell Pellet

- B2. Discard 100% Supernatant
- B3. Reconstitute Cells into 1.7ml 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. 10.2ml.

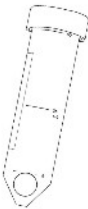
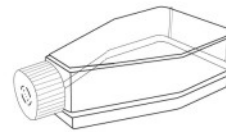


Plate Cells



- B4. Transfer 10.2mls of Reconstituted Cells in a Pre-coated T75 Flask
- B5. Incubate at 37°C, 5% CO₂ 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. 10ml per T75.

Note: If the attached Cells density is low, combine with Suspension Cell Pellet into a total volume of approx. 10ml per T75 Flask.