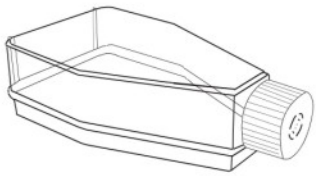
Seeding Protocol

CELPROGEN™
Stem Cell Research & Therapeutics

Plated Cells



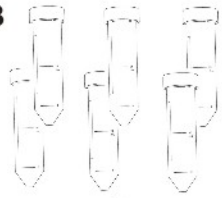
1. Wipe down flask with 70% Alcohol upon arrival.
2. Transfer in to Tissue Culture Hood

Inside Tissue Culture Hood

Original Flask **A**

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

B



Attached Cells in Flask

Original Flask

A1. Wash with 3ml 1XPBS 2-3min remove and discard 100% 1XPBS Cat# P1408-013
A2. Incubate at 37°C, 5% CO_2 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.

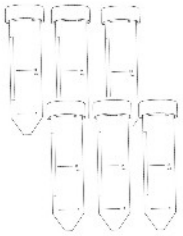
A9. Discard 100% Supernatant
A10. Reconstitute Cells in 10ml of Complete Growth Media

Cell Pellet

A11. Transfer 10mls of the Reconstituted Cells in a Pre-coated T75 Flask
A12. Incubate at 37°C, 5% CO_2 in Humidified incubator.

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

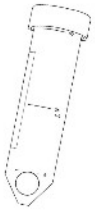
Suspension Cells



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% CO_2 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.