ProMab Biotechnologies, Inc.

Protocol

# Generating a Single Cell Suspension from Adherent Cells

## OVERVIEW

Specific cell culture procedures, and products to allow for Cancer Stem Cell isolation and propagation as tumor spheres.

## DISRUPTION OF ADHERENT CELL PROCEDURE

Starting from a confluent (90%) culture in a T75 tissue culture flask

1. Remove existing medium, and rinse adherent cells with 5 mL of sterile Hanks Balanced Salt Solution (HBSS), or Phosphate Buffered Saline (PBS); discard wash (this will remove trace amounts of fetal bovine serum (FBS), which will normally may inhibit the action of trypsin).
2. Add 5 mL of pre-warmed (37°C) Trypsin:EDTA, and place the flask in a 37C incubator for 2 min.
3. Following incubation cells should be loosely attached to the flask surface, and cells are disrupted by repeated pipetting (using a 5 mL sterile pipette).

*The Trypsin: EDTA incubation time is critical to avoid excess clumping and cell death. While 2 min will work for many cell types/lines, this may need to be optimized based on the specific cell type/line being used.*

4. Following disruption, immediately add 15 mL of DMEM media, containing 10% FBS, to the trypsinized cells, transfer to a sterile 50 mL polypropylene conical centrifuge tube, and pellet cells by centrifugation at 1000 RPM for 5 min.
5. Resuspend cells in 10 mL of Cancer Stem Premium™ (cat. # 20101, ProMab Biotechnologies) media.
6. The cell suspension is passed through a sterile 20 µM cell strainer to remove any cell clumps remaining in the cell suspension.
7. Remove a 10 µL aliquot for cell counting.




2600 Hilltop Drive, Richmond, CA 94806 | Tel:1-866-339-0871 Fax: 510-740-3625 | www.promab.com | info@promab.com