



1. Thawing Suspension cells
 - a. Prewarm complete medium to room temperature (not 37°C) and transfer 8ml of it to a 15ml tube; transfer another 10ml to a T25 flask
 - b. Quickly thaw the cryovial at 37°C (a small ice clump should still remain and the cryovial should still be cold)
 - c. Transfer the complete content of the cryovial to the 8ml of media and resuspend well
 - d. Spin the cell suspension at 300xg for 3min and discard the supernatant carefully
 - e. Take 5ml out of the T25 flask and resuspend the cell pellet in the 15ml tube. Take a small aliquot of cells (<300µl) to count the cells and determine the viability.
 - f. Transfer the rest of the cell suspension to the T25 flask, resuspend well with the remaining 5ml of media.
 - g. Incubate at 37°C for at least 24 hours

2. Subculture Suspension cells
 - a. The cells should not exceed a cell density of 1×10^6 cells/ml.
 - b. Carefully transfer the cell suspension to a 15ml tube and centrifuge at 300xg for 3min.
 - c. Aspirate the supernatant of the cells and resuspend the cells in 5 or 10ml of medium (depending on the cell pellet). Take a small aliquot to count the cells and determine the viability.
 - d. Seed the cells at a density of 1×10^5 cells/ml in either T75 flasks or T150 flasks. Transfer 15ml of fresh medium to each T75 flask (or 30ml for each T150 flask) and dispense the cell suspension equally to the T75/T150 flasks.

3. Freezing Suspension cells
 - a. Carefully transfer the cell suspension to a 15ml or 50ml tube and centrifuge at 300xg for 3min.
 - b. Aspirate the supernatant and resuspend the pellet in a defined volume of medium (e.g. 5ml, 10ml, 20ml; depending on the size of the cell pellet). Take a small aliquot to count the cells and determine the viability.
 - c. Depending on the cell count, determine the amount of cryovials.
 - d. Centrifuge the cell suspension at 300xg for 3min and resuspend the cell pellet in the calculated amount of CM-1 (freezing medium).
 - e. Store the cryovials at -20°C immediately for at least 40min. Transfer all cryovials to -80°C overnight. Prolonged storage must be in liquid nitrogen (-196°C).