1. Thawing adherent 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 with the other 5ml of media and transfer 5ml of cell suspension to a second T25 flask
    g. Incubate at 37°C for at least 24 hours

2. Subculture adherent cells
    a. You should subculture the cells at an 80% confluence.
    b. Carefully aspirate the supernatant of the cells. Wash once with 2.5ml (T25 flask) or 5ml (T75 flask) 1xPBS (without Ca and Mg). Add 1ml (T25 flask) or 2.5ml (T75 flask) Accutase (available at CLS) to the cells and incubate at 37° for 10min.
    c. Add approximately 3ml of fresh medium to the cells and resuspend the Accutase/Medium mixture. Transfer the mixture to a fresh 15ml tube.
    d. Centrifuge the cell suspension at 300xg for 3min, aspirate the supernatant and resuspend the cell pellet in 5ml of fresh medium. Take a small aliquot to count the cells and determine the viability.
    e. Seed the cells at a density of 1x10^4 cells/cm^2 in either T75 flasks or T150 flasks
    Example: 1.6 x 10^6 cells/ml were counted
    1.6 x 10^6 Z/ml = 16 x 10^5 Z/ml
    16x 10^5 Z/ml x 5ml = 80 x 10^5 Z/5ml
    80 x 10^5 Z/5ml / 7.5 = 10.66 (number of new T75 flasks)
    f. Transfer 15ml of fresh medium to each T75 flask and dispense the cell suspension equally to the T75 flasks.

2. Freezing adherent cells
    a. You should freeze the cells at an 80% confluence.
    b. Carefully aspirate the supernatant of the cells. Wash once with 2.5ml (T25 flask) or 5ml (T75 flask) 1xPBS (without Ca and Mg). Add 1ml (T25 flask) or 2.5ml (T75 flask) Accutase (available at CLS) to the cells and incubate at 37° for 10min.
    c. Add approximately 5ml of fresh medium to the cells and resuspend the Accutase/Medium mixture. Transfer the mixture to a fresh 15ml tube/50ml tube depending on the volume of the total cell suspension. Centrifuge the cell suspension at 300xg for 3min.
    d. 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.
    e. Depending on the cell count determine the amount of cryovials.
    f. Centrifuge the cell suspension at 300xg for 3min and resuspend the cell pellet in the calculated amount of CM-1 (freezing medium).
    g. 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).