Thawing protocol

- Swirl cells in water bath
- Add 1mL of warm medium to fully thaw the cells
- Homogenise
- Transfer the suspension to 15mL tubes prefilled with 3mL medium
- Centrifuge 5min at 1500 rpm
- Discard the supernatant
- Transfer to T75 flask
- Put in the incubator, 37˚C and 5%CO_2

Cells should stay in the incubator for 2-3 days. You should check every day how confluent they are and change the medium every two days. When they are around 75-80% confluent, you can either split them, plate them for experiment or freeze in -80.

Cell passaging/Splitting

- wash cells with 5mL of PBS
- add 2mL of trypsin
- incubate 1-2min at 37˚C
- check the cells under the microscope
- add 5mL of medium and pipette up and down to detach cells
- centrifuge suspension and discard the supernatant
- resuspend cells in 6mL in a tube
- dispense 10mL of fresh medium into new T75 flask
- transfer 1mL from the old flask to a new one (adjust according to cell number)
- incubate at 37˚C

Cell freezing

- rinse cells with 5mL of PBS
- add 2mL of trypsin to detach cells (put them in the incubator for 1-2min)
- add 4mL of medium and homogenise
- centrifuge the cells 5min at 1500 rpm
- discard supernatant
- resuspend the cells in 1.5mL medium and 1.5 mL freezing medium
- transfer 1mL into cryovial (3x 1mL)

  Freezing medium: 80%FBS* + 20%DMSO