

Splitting

1. Take a look at the cells and see that they are alive and well.
2. Heat ~3 ml trypsin (15ml falcon in the freezer) in a 37⁰c bath for ~10 minutes.
Write the date on the falcon.
3. Heat ~22 ml DMEM medium (14 ml for the flask, 6 ml for inactivation of trypsin, 2 ml extra) in a 37⁰c bath for ~10 minutes.
4. Aspirate old medium and immediately add ~3 ml trypsin and incubate for ~3 minutes in 37⁰c.
5. In the meantime, add 14 ml medium to a new flask.
6. After ~3 minutes, take a look at the cells and check if they detached. If not, continue incubation.
7. Add 6 ml medium to the old flask with the trypsin.
8. Take 1 ml from the old flask and move it to the new flask.