

Thawing cells:

1. Place DMEM bottle into the water bath.
2. Clean bench with EtOH.
3. Sterilize DMEM bottle and hands with EtOH.
4. Flame DMEM bottle:
 - a. Cap on bottle
 - b. Bottleneck without cap
 - c. Cap
5. Take a 75-bottle, label it with cell type, date, name, passage.
6. Fill 20 mL DMEM into the 75-bottle.
7. Fill 10 mL into a 15 mL- falcon. (Don't forget labelling)
8. Fetch cells from the -80°C freezer and place them into the water-bath for max. 2 minutes.
9. **Hurry up with the following steps!** (DMSO is toxic for the cells.)
10. Check the passage before you sterilize the eppi with EtOH.
11. Adjust the pipetto pipette-speed to minimum.
12. Add the cell suspension to the 10 mL DMEM in the 15 mL falcon.
13. Centrifuge: 200 g / rcf for 5 minutes
14. Check if there's a pellet.
15. Remove supernatant with a 5 mL-pipette.
16. Take ~ 3-5 mL of the DMEM medium of the 75-bottle, resuspend pellet and fill cell suspension into the 75-bottle.
17. Place cells into the incubator (horizontal!)
18. Sterilize bench with EtOH.