freeze cellculture

- Switch the water bath (37°C) on, **45 minutes before you start working** at the bench and heat up the cellculture medium and the PBS in the water bath.

- Switch the UV-light and the ventilation on, **30 minutes before you start working** and switch the UV-light off as soon as you start working.

- Always wear gloves and spray them with EtOH before working at the bench or before you take sth. out of/put sth. into the cellculture cupbaord.

- Before you start working spray the bench with EtOH and wipe away the remaining EtOH with paper towels after waiting for a few seconds.

- Spray all flasks (excluding the cellculture flasks) with EtOH , wait for a few seconds then remove the remaining EtOH with a paper towel and place it on the bench.

- Flame all flasks shortly before you open it (excluding the cellculture flasks).

Take the 10 cm cellculture dishes carefully out of the cupboard and screen it for density, dead cells and bacteria at the microscope. Then, place the dish on the bench, remove the medium (don't dry the cells out, hurry !) and wash carefully with PBS (10 cm cell culture dish: 8 ml; T75-flask: 10 ml), then remove the PBS.

Pipet Trypsin (EDTA in solution) (10cm dish: 0,8 ml; T75-flask: 1ml) to the cell culture and sway it carefully (30-60 s) to detach the cells from the dish/flask. Then, pipet DMEM (contains FCSI which inhibits Trypsin) onto the cells (10 cm dish: 5-8 ml; T75-flask: 10 ml).

Pipet the cellculture into a 15ml falcon and centrifuge (3 minutes, 200G) and then label the cryo-tube (celltype, passage, your name, date). Discard the supernatant and resuspend the pellet with 1,5 ml DMSO-Cryomedium (shake before apply).

Pipet the resuspended cells into the cryo-tube and put it into the isopropanol-container (-80°C coupboard). Take the cryo-tube out of the isopropanol-container and put it on its place **24 h later**.