

split 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 cupboard.
- 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 out the cells, 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 medium and the cells into a 15 ml falcon and centrifuge (200 G, 3 minutes).

Label the new flask (cell-type, passage, name, date) and submit 20 ml fresh cell culture-medium into the new flask during these 3 minutes.

Discard the supernatant (after centrifugation) and pipette 5ml from the T75 flask into the falcon and resuspend the cells. Attention: maybe the pellet is loose so be careful.

Then, pipet x ml (depends on cell density and further working steps) of the resuspended cell into the new flask.

Take care that the lid doesn't contact the liquid because it clogs the membrane within the lid

=> the cell die

Hold the flask vertically and shake it a little bit then put it into the cellculture cupboard (horizontally).