SDS-PAGE

Aim:

- Identifying the size of the protein of interest

Timeframe

- 60 minutes

Materials

- Purified protein sample
- LAEMMLI 2x concentrated containing mercaptoethanol
- 1 x SDS running buffer containing 25 mM Tris, 190 mM Glycine and 0.1 % SDS
- MilliQ water
- Protein ladder
- Gel tank
- Power supply
- Weigh boats
- Instant blue

Procedure

1. Prepare an eppendorf tube with 5 uL of purified protein, 5 uL of 2x LAEMMLI with mercaptoethanol and 10 uL of milliQ water.
2. Place the eppendorf tube in the thermocycler and incubate at 95°C for 5 minutes..
3. Remove it from the thermocycler.
4. Remove white strip and comb from a denaturing precast gel.
5. Place the gel in the tank making certain that the wells face inwards and the gel is sealed tight in place (ask a supervisor to help).
6. If only one gel is run place a blank gel cassette on the opposite side to form a closed chamber.
7. Fill the tank with buffer starting from the enclosed area of the gel and then filling it all up, in the meantime, ensure that no leakage is occuring.
8. Plug the cables in the power box and run the gel at 180 V for 35 minutes until the running front reaches the bottom of the gel.
9. When the gel has finished running, remove the gel from of the tank, break the plastic sealed cassette and place the gel into a plastic box.
10. Pour instant blue stain on the gel and leave it to stain for a few hours.
11. Remove the stain and place in water, ready to be visualised in a transilluminator.