Taking samples from *E. coli* cultivations for SDS-PAGE

This sampling will normally include all proteins within the cell, both soluble proteins and proteins that may be in an insoluble/aggregated form. This sampling method normalizes the cell concentration, which is suitable for following expression of a specific protein, like a recombinant protein.

1. For each sampling point, two samples are taken from the culture. One for measuring OD, and one for SDS-PAGE. Both samples are needed in order to normalize the cell concentration in the SDS-PAGE samples.
2. Prepare disposable cuvettes with water for suitable dilution of the cultures. 10 times dilution (900 µL water + 100 µL cultivation) normally works OK for the first part of the cultivation. When the measured OD increases above 0.5-1.0 further dilution is needed (e.g. 950 µL water + 50 µL cultivation). Also prepare small pieces of parafilm for mixing of cuvette content before measuring.
3. Prepare a labeled 1.5 mL tube for the SDS-PAGE sample.
4. Take out the culture from the incubator. Mix the culture just before taking the samples. The sample for the OD measurement and transfer directly to the cuvette with water. Make sure the sample is actually taken from the culture liquid and not from any foam that may be present. Also make sure the outside of the pipette tip is free from culture droplets before transfer to the cuvette.
5. Take another sample from the culture and transfer to the Eppendorf-tube. For most part of the cultivation (OD below ~10) a 500 µL will work well. For higher ODs 250 µL will be sufficient. “Too large” volume will… ???
6. Put the culture back in the incubator if the cells should be cultivated any further.
7. Measure OD600 in the cuvette. Before putting the cuvette into the spectrophotometer, place a piece of parafilm over the cuvette and mix the content by inverting the cuvette a few times. Calculate the OD in culture.
8. Centrifuge the Eppendorf tube at 8000 rpm for 1 minute in the microcentrifuge. Carefully remove and discard the supernatant. Using on the culture OD, calculate the volume needed to suspend the cell pellet in order to obtain OD = 10 (use the formula: c1 x V1 = c2 x V2).
9. Use the pipette to suspend the cell pellet in the appropriate volume of SDS-PAGE loading dye to give OD = 10.
10. Boil the sample in the heating block at 100°C for 5 minutes. Use the safe cap to prevent the lids from popping up.
11. Store samples at 4°C for a few days, or -20°C for longer storage.
12. 5 µL sample normally gives a good band intensity on a 15 well SDS-PAGE gel.

Protocol summary

1. Take one sample for OD measurement (to cuvette) and one for SDS-PAGE (to Eppendorf tube).

2. Measure OD600.

3. Centrifuge the Eppendorf tube at 8000 rpm for 1 minute. Remove supernatant.

4. Suspend cell pellet in SDS-PAGE loading dye to OD = 10. Boil 5 minutes at 100°C.