Absorbance Spectrometry

GROWTH CURVE DAY 1

Prepare overnight cultures of your samples:
Use autoclaved 100ml flasks, mark flasks and find plates with appropriate colonies.
Add 10 ml media (TSB/LB) to the 100ml flasks.
Add 1x of chloramphenicol (1000x stock), (except to top10 wt bacteria)
(If the brick contains pBAD) Add 1x of Arabinose (50x stock) (1x is equal to 0.2% Arabinose)
Pick a colony from the plate using an inoculation loop, put the loop in the flask and stir it.
Cover the flask and leave at 37°C overnight.

GROWTH CURVE DAY 2

Bring overnight cultures to the bench.
You need to dilute samples 1:10 (in media) to read the OD: Prepare UV-cuvettes. Add 900ul of media to all sample cuvettes and include a blank cuvette with 1000ul sterile media. Add 100 ul of sample from the overnight flasks into the sample cuvettes. Shake the cuvette carefully. Measure OD of all the samples and multiply the OD by 10.
Make the calculation: OD/0.2=X ---> 20/X= Y --> Y= the amount (in milliliters) to add to the 20 ml flask to dilute cells to OD 0.2
grow until O.D: 0.6-0.8.
Prepare new 100 ml culture flasks with 30 ml media, 1x chloramphenicol and 1x arabinose to relevant flasks.
Dilute overnight cultures to OD 0.05 in 30 ml.
When the samples are added to the media - time zero has begun. Put the culture flasks at 37°C shaking.
Grow samples for 5h, that is to 300 min, measure the OD every 15 min.
Use sterile serological pipettes to retrieve samples from the flasks. Wash your cuvette with deionized water after each OD measurement.
When OD approaches OD 0.800, dilute samples 1:2 in the cuvette before measuring.
Collect 2 ml of samples for Western Blot at time: 150 min, 180 min, 210 min and 300 min.

ALTERNATIVE CURVE
Inoculate cells in 10 ml TSB with 10ul Chloramphenicol, grow overnight 37°C. Dilute overnight cultures to OD 0.2 in 10 ml TSB with 10ul Chloramphenicol and grow until OD 0.6. Dilute cultures to OD 0.05 in 30 ml TSB with 30 ul of chloramphenicol (and 0.2% Arabinose) and grow for 5 hours.
Take samples (1.5 ml) at 6 time points.