iGEM2019 Protein prep protocol

TCA Extraction

1. Inoculate cells
2. Dilute cells to OD: 1-2 in 2mL eppendorf tube
3. Spin cells at 3K rpm, room temperature (RT) for 3 minutes.
4. Remove supernatant.
5. Set a centrifuge to temperature 4◦C.
6. Collect ice in a box.
7. Flash freeze tubes in liquid nitrogen. After this, the cells can be stored at -80 ◦C.
8. Keep the tubes on ice.
9. Add 300 uL of cold TCA buffer to the frozen pellets.
10. Let sit on ice until fully thawed. Mix briefly.
11. Spin at 4◦C, 16K rcf, for 10 min.
12. Remove supernatant.
13. Put heating block to ~98◦C.
14. Wash: Add 500 uL of cold 80% acetone. Vortex.
15. Spin at max speed at 4◦C, for 5 min.
16. Remove supernatant.
17. Ad 75 uL Resuspension Solution.
18. Boil samples for 10 min on heating block. - Here you can start preparing the qubit protein assay, just don't add the Qubit protein reagent yet.
19. Allow to cool down in RT.
20. Spin down cell debris for 1 min, at RT, max speed.
21. Transfer 60 uL of supernatant to new eppendorf tube.
22. Measure protein concentration using Qubit Protein Assay. Instructions for this can be found bellow.
23. Dilute the protein in MQ water, in a fresh tube, to a total volume of 60 uL and a total protein concentration of 2ug/uL.
24. Add 15 uL of 4X SDS PAGE loading buffer, and 7.5 uL of 1M DTT.
25. Boil the samples for 5 minutes, and spin them down briefly.
26. Store samples at -20◦C for short term storage, and at -80◦C for long term storage.

Qubit Protein Assay

1. Prepare 3 standards (S1, S2, S3). These can be saved for later use.
   The standards need to have BSA concentrations according to the table below.
   Prepare a working solution of BSA of 2ug/uL. Store solution at 4◦C. It can be stored for a few months.

Label	Final BSA concentration (ng/uL)	BSA (uL)	TE (uL)	Resuspension Solution (uL)
S1	0	0	95	5
S2	200	10	85	5
S3	400	20	75	5

2. Label Qubit tubes for the standards and your samples.
3. Add 10 uL of the standards to Qubit tubes.

4. Add 9.5 uL of TE to your sample Qubit tubes.
5. Add 0.5 uL of samples to your sample Qubit tubes.
6. Make Working Solution. Keep it in a foiled tube, since it is light sensitive.

Working Solution:
N*199 uL of Qubit protein buffer
N*1 uL of Qubit protein reagent - this is light sensitive. Don't work to slow. :)

7. Add 190 uL of Working Solution to every Qubit tube.
8. Vortex briefly. Try to avoid foam.
9. Incubate at RT for 15 minutes. (tip: desk drawer)
10. Read flourescence on Qubit Fluorometer:
a. Select Home screen
b. Select Protein
c. Start with making a standard curve by reading S1, S2 and S3.
d. Set the sample volume to 5 uL. (Then you can multiply your results by 10 to get your actual concentration)
e. Read samples