Flow Cytometry using SITOX Green[1]

Prepare solutions, some needs boiling
70% EtOH in -20ºC

1. 0.5ml GPY of culture, grow overnight.
2. $1x10^7$ cells are harvested by centrifugation, 2000rpm 5min. (First day)
3. Wash with 1ml dH_2O.
4. Add 1ml of cold 70% EtOH, with slow mixing to fix the cells.
5. Fixation 1h at RT or overnight at 4ºC. (Second day)

Wait until 17:00

Turn on bath at 37ºC

7. Centrifuge and wash once in 1ml of Tris Buffer.
6. Sonicate 3x for 5-10 seconds (depending on the floculation character) at low power, put on ice each time.
8. Centrifuge and resuspend in 0.5ml RNAse solution
9. Incubate in RNAse solution for 6h (o/n) at 37ºC. Keep at 4ºC o/n. (Third day)

Thaw SYTOX aliquotes

10. Centrifuge and resuspended directly in 0.2ml protease solution.
11. Incubation for 15-20min at 37ºC.
12. Centrifuge and resuspend in 0.5ml 50mM Tris pH 7.5 (we can store at 4ºC for few days or analyzed immediately).
13. 50µl of cell suspension is placed into 1ml of SYTOX Green solution.
14. Analyze by standard flow cytometry method.

    Argon laser 488nm

    FL1 detector with standard 530/30 band pass filter

Solutions (for 50 measurements)

RNAse solution (25ml) Cat. No. 12091-021
2mg/ml RNAse A in 50mM Tris pH 8.0, 15mM NaCl
Boiled 15min and allowed to cool at room temperature

Stock RNase A: 20mg/ml
Stock Tris HCl: 1M Tris HCl pH 8.0
50mM Tris HCl → 2.5ml Stock Tris + 47.5 ml dH_2O

Method A (RNase in solution)
2.5ml (50mg) of RNAse A + 21.915mg of NaCl + 22.5ml 50mM Tris pH 8.0 (keep +4ºC)
Method B (RNase in powder)
Boil RNase solution for 15min and allow to cool at room temperature.

Protease solution (10ml) (Fresh made)
5mg/ml pepsin, 4.5µl/ml concentrated HCl, in H_2O.

50mg pepsin + 45µl of 37% of HCl in a final volume of 9.45ml in H_2O.
Disolve pepsin in water before add HCl

SYTOX Green solution (50ml) (store in the dark -20ºC)
1µM SYTOX Green in 50mM Tris pH 7.5

10µl SYTOX Green 5mM in final volume of 50mL of 50mM Tris pH 7.5
To aliquot in 1mL final volume

1. Haase, S.B. & Reed, S.I. Improved flow cytometric analysis of the budding yeast cell cycle. *Cell cycle (Georgetown, Tex.)* **1**, 132-6 (2002).