

Flow Cytometry for Yeast

1. Remove 1mL of cells from liquid culture at 0.4 to 1.0 OD600 and put into an eppendorf tube (be sure to always include an asynchronous wild-type haploid culture to calibrate the flow cytometer).
2. Spin down the cells in a microfuge.
3. Aspirate off the supernatant.
4. Add 1-1.5mL of 70% EtOH and resuspend the pellet by vortexing (the exact amount is not critical, so you can just squirt some in from the squirt bottle).
5. Let the cells sit at room temperature for at least 1 hour, then store at 4 degrees or continue with the protocol.
6. Spin down the cells in a microfuge and carefully aspirate off the supernatant.
7. Resuspend the cells in 1-1.5 ml FACS buffer (again, the exact amount is not critical, so just squirt some in from the squirt bottle)
8. Spin down the cells and aspirate off the supernatant. Repeat wash with FACS buffer.
9. After 2nd wash, resuspend in 100µl of RNase diluted 1:10 in FACS buffer (10µl 1% RNase + 90µl FACS buffer; make up a mix for all your samples).
10. Incubate the cells at 37°C for 2 hours to overnight.
11. Spin down the cells and aspirate off the supernatant.
12. Resuspend the pellet in 1-1.5 mL PBS (again, the exact amount is not critical so just squirt some in from the squirt bottle)
13. Spin down the cells and aspirate off the supernatant.
14. Resuspend the pellet in 100µl of propidium iodide solution diluted 1:10 in PBS (10µl 0.5 mg/ml propidium iodide + 90µl PBS; make up a mix for all your samples).
**wear gloves because propidium iodide is not good for you!!
15. Let sit from 1 hour up to overnight in the dark in the fridge.
16. Bring the volume up to 1mL by adding 900µl PBS to each tube.

17. Sonicate the samples for 10 sec. at 30% power.

18. Run samples immediately on the Flow Cytometer (take them down to cytometry).

5X FACS Buffer

1 M Tris-HCl pH7.5

100 mM EDTA

To use: add 100 ml 5X FACS Buffer and 5 ml 2% NaN₃ to 400 ml ddH₂O, and put into 500 ml squirt bottle

1% RNase A (10X)

stored at -20°C

0.5 mg/ml propidium iodide (10X)

stored at 4°C

10X PBS

To use: add 50 ml 10X PBS, pH 7.4 and 5 ml 2% NaN₃ to 450 ml ddH₂O, and put into 500 ml squirt bottle

70% EtOH

Put 370 ml 95% EtOH and 130 ml ddH₂O into 500 ml squirt bottle