
Yeast Cell Cycle by Flow Cytometry

Reagents

* Cold absolute ethanol.
* 10 mM EDTA pH 8.0 (filtered).
* 10 mg/ml RNase A (Boil 10 mins, cool, filter and store at -20°C).

* SYTOX Green (Molecular Probes catalog S-7020; 5mM stock in DMSO and stored in dark at -20°C)

Protocol

1. Spin down 107 cells from an exponentially growing culture - 2000 rpm for 5 mins. Pour off supernatant.

2. Vortex tube while adding 1.0 ml cold 70% EtOH.
    - Store at 4 °C (cells keep ~indefinitely).

3. When you want to process the cells, take 0.3 ml (this will be 2-3 x 106 cells, assuming a little loss in the washing) and add to 4.5 ml 10 mM EDTA pH 8.0 in a 5ml Falcon tube. Mix and spin 2000 rpm for 5 mins.

4. Discard supernatant and resuspend pellet in 0.5 ml 10 mM EDTA pH 8.0 containing 0.1 mg/ml RNase A. Leave in 5 ml Falcon tube and put in 37 °C room for 2 h.

5. For staining:

    - Sytox Green

    Add 0.5 ml 10 mM EDTA pH 8.0 containing 2 µM Sytox Green, so that final concentration in the sample is 1 µM.

6. (Optional) Just before processing the cells, sonicate for 45 s again leaving cells in the 5 ml Falcon tubes. Sonication prevents doublets of cells which give spurious peaks and is particularly useful if your cells have varying DNA contents and will clean up spores or wee mutants.

7. Approximate settings on the FACScan for Propidium Iodide
    - Detector FSC E00 Gain:3
    - Detector FL2-A Voltage:890 Gain:2

8. Approximate settings on the FACScan for Sytox Green
    - Detector FSC E00 Gain:2
    - Detector FL1-A Voltage:400 Gain:4

Points to bear in mind

* You can fix more than 107 cells, but don't process many more than 5x106 fixed cells. Using too many cells can lead to incomplete staining and artefacts.
* You can make controls representing 1, 2 and 4C DNA contents. Use nitrogen starved haploid cells, exponentially growing haploids and exponentially growing diploid cells respectively. You can fix large

numbers of cells and use them over many months. It's helpful to include a control sample in each series of samples that you process.

* Ethanol fixed cells can be sent in the post at room temperature without coming to any harm. Stained cells can be FedEx'd without coming to any harm.
* If you are dealing with particularly fragile cells (e.g. very elongated cells) there may be a problem with lysis when cells are washed in water before fixation. This can be avoided by washing with 1M sorbitol. You can even fix cells in 70% ethanol, 30% 1 M Sorbitol. If you have problems with lysis even in the culture medium, then 1.2 M sorbitol can be included here as well. Wash out the sorbitol before flow cytometric analysis because it destabilizes the sample stream resulting in high CVs.
* Learn how to use the 'Live Gate' option. This allows you to reduce the background in your samples (which may be caused by anything from particles of medium to bacteria or other contaminants) and will improve your data. It also gives you the option of focusing on a particular subpopulation that you may be interested in.

General reference (PI method) : Sazer and Sherwood (1990) J. Cell Sci 97:509-516

--> Pombe methods page
--> Salk flow cytometry home page

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