

Cell Cycle Analysis by Propidium Iodide Staining

Background

This is a method for cell cycle analysis using propidium iodide (PI), that is, using the fluorescent nucleic acid dye PI to identify the proportion of cells that are in one of the three interphase stages of the cell cycle. Please take into account the following when using this method:

1. This method works well to assess cell cycle distribution of a whole cell population when cells are fixed with 70% ethanol.
2. This method can also be used to assess cell cycle distribution of certain GFP transfected cells. The ethanol fixation step is generally not suitable to keep GFP in the cell. The use of membrane localized GFP fusion protein (i.e. **Spectrin**) provides excellent results employing this ethanol fixation procedure and is highly recommended to assess the cell cycle distribution in GFP transfected cells. Otherwise consider a live cell DNA stain such as Hoechst 33342 or Draq5. Aldehyde fixation (e.g. PFA), which cross links cellular protein together and hence keep GFP inside the cell is not suitable for cell cycle phases analysis without further optimisation (see “Internal antigens and cell cycle” protocol).

Materials

1. 70% ethanol (in DI water)
2. 1x PBS
3. PI solution in PBS (50µg/ml)
4. RNase A solution in PBS (100µg/ml)

Equipment

1. **Centrifuge.**
2. **Pipettes.** You will need one in the range of 2-10µl, one in the range of 10-100µl, and another ranging from 100-1000µl.
3. **Vortex mixer.** You *could* mix by tapping or shaking the tubes, but a mixer will give much more reproducible results in most cases.
4. **12x75 mm polystyrene/polypropylene tubes.** Depending on which machine you wish to use (LSR II prefers polystyrene while the CyAn prefers polypropylene).
5. **Ice bucket with cover.** Generally, cells are more stable and tolerate insult better when they're cold. The cover keeps light out, which could bleach the fluorochromes.
6. **Flow cytometer.** We have a variety of machines at your disposal including a BD LSR II, BD FACSAria and a Beckman Coulter CyAn. Each machine has different capabilities, strengths and weaknesses so be sure to check with us which machine is suited for your needs.

Procedure

1. Harvest cells in the appropriate manner and wash in PBS.

2. Fix in 1ml cold 70% ethanol. Add drop wise to cell pellet while vortexing. This should ensure fixation of all cells and minimise clumping.
3. Fix for at least 30 minutes on ice. Specimens can be left at this stage for several weeks (make sure you seal the tubes for long term storage).
4. Pellet cells at higher speed compared to live cells for 5 minutes, aspirate the supernatants being careful not to lose the pellet. Note that ethanol-fixed cells require higher centrifugal speeds to pellet compared to unfixed cells since they become more buoyant upon fixation.
5. Wash twice with PBS.
6. To ensure that only DNA is stained (PI stains all nucleic acids), treat cell pellet with Ribonuclease A to get rid of RNA. Add 50µl of RNase A solution directly to pellet.
7. Add 400µl PI solution per million cells directly to cells in RNase A solution. Mix well.
8. Incubate cells for 5 to 10 minutes at room temperature. Some cells (e.g. fibroblasts) may need longer incubation period, usually overnight, to stain properly.
9. Analyse samples by flow cytometry in PI/RNaseA solution (no need to wash cells). Save at least 10,000 single cells.

Flow analysis:

Keep the cells at RT covered until your scheduled time on the flow cytometer.

When analysing samples, be sure to collect PI in a linear scale. Use a dot plot showing PI parameter Area vs Height (LSRII)/Peak (CyAn) or Width (LSRII) to gate out doublets and clumps and analyse at a low flow rate under 400 events/second.