

Cell Cycle Analysis by Propidium Iodide (PI) Staining

Adherent cells:

1. trypsinized
2. suspended in medium + 10% FCS
3. centrifuged (1000 rpm, 5 min)
4. Pellet suspended in PBS (1 ml)

Suspension cells:

1. Centrifuged (1000 rpm, 5 min)
2. Pellet suspended in PBS (1 ml)

Fixation with EtOH:

Pipet cell suspension into 2.5 ml absolute EtOH (final concentration approx. 70%) - or vortex the suspension at half speed while adding the EtOH) – to prevent clustering of cells during the fixation. Incubate on ice for 15 min (or over night at -20°C).

Alternative fixation with paraformaldehyde:

Pipet the 1 ml cell suspension into 3 ml 4% paraformaldehyde and fix for 15 min at r.t.

Staining:

1. Pellet the cells at 1500 rpm for 5 min
2. Suspend the pellet in 500 μl PI-solution
in PBS: 50 $\mu\text{g}/\text{ml}$ PI from 50x stock solution (2.5 mg/ml)
0.1 mg/ml RNase A
0.05% Tritin X-100
Incubate for 40 min at 37°C
3. Add 3 ml of PBS, pellet the cells (1500 rpm, 5 min) and take off the supernatant
4. Suspend the pellet in 500 μl PBS for flow analysis
(you can also leave about 500 μl of the diluted staining solution on the pellet and suspend the cells in this solution > less loss of cells when you take off the sup.) – the rest of the staining solution does not interfere with the flow analysis.

Flow analysis:

Approximate settings (on FACSort):

FL1: 570 V log. (e.g. if you want to detect GFP)

FL2: 470 V linear

Example:

