Cell cycle analysis – Propidium iodide (PI)

1. Harvest approx. $1\text{^}10^6$ cells and centrifuge at 400 g for 5 min
2. Wash cells once with cold PBS
3. Resuspend and fix the cells by adding 0.5 ml ice-cold 70 % ethanol dropwise while slowly wortexing to prevent clumping
4. Incubate on ice for more than 2 hours. At this point the cells can be stored for weeks (4˚C or -20˚C)
5. Spin down the cells and wash twice with PBS + 1% FBS (600 g and be careful to avoid cell loss when discarding the supernatant especially after ethanol treatment)
6. Resuspend cells in a 1 mL mix of 200 µg/mL RNAse and 50 µg/mL PI in PBS and incubate for 30 min at +37˚C protected from light
7. Do NOT wash the cells prior to analysis on the flow cytometer. PI is an intercalating agent and will leak out of the cell if the population is resuspended in buffer not containing PI
8. Analyze your cells with flow cytometer using a slow flow rate. Make sure the flow stays approx. 200 events/s.
9. Measure the forward scatter (FFC-A) and side scatter (SSC-A) to identify single cells. Pulse processing is used to exclude cell doublets from the analysis. This can be done either by using pulse area vs. pulse width or pulse area vs. pulse height (depending on the type of flow cytometer)

NOTE

- 70% ethanol should not be made with PBS as this causes protein precipitation on fixation. Use 70 parts absolute ethanol to 30 parts distilled water
- PI is a carcinogen and should be handled with care. It also stains RNA, so RNAse treatment is needed for cell cycle analysis
- As PI cannot cross the intact cell membrane, it can be used as a viability dye. PI enters only the cells with damaged plasma membrane