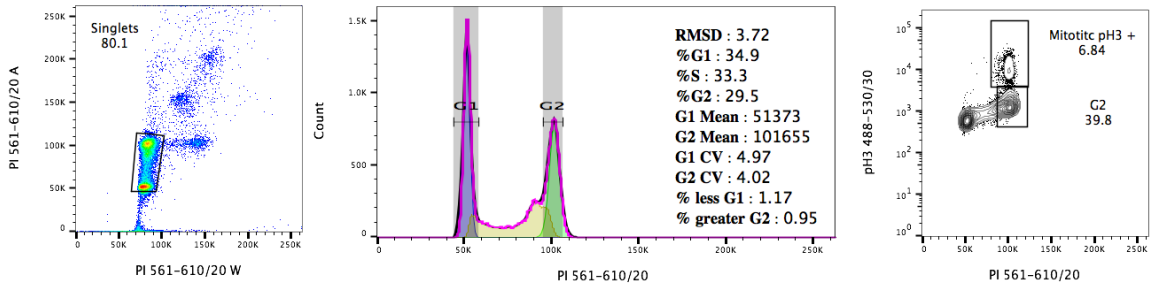
Propidium Iodide and Phospho-Histone H3 (Ser10) Staining

The critical event occurring during the G2 to M transition is the phosphorylation of histone H3 at Ser 10, which appears to be essential for chromatin condensation. Since the time interval during which histone H3 remains phosphorylated is restricted to mitosis, this event constitutes a specific marker discriminating mitotic cells. The use of antibody that specifically recognizes only H3 phosphorylated at Ser 10 (anti-H3-P mAb) provides the means for detection of H3 phosphorylation, and thereby the detection of mitotic cells. When a DNA dye is added to the assay, the proportion between G2 and Mitotic cells could be determined as well as the population of cells in G1 and S pase.



Data from Elisabeth Zielonka/Hentze Group

For a better detection of pH3 positive events, cells are incubated with nocodazole which interfere with the polymerization of microtubules, arresting the cells in G2/M phase.

Protocol:

1. Apply the desired treatment to the cells (drug exposition, DNA damage induction, etc.)
2. Wash the cells and put fresh medium containing nocodazole (100ng/ml), keep the cells in culture for the time established in your protocol.
3. Remove cells from the culture plate and spin at 1200rpm for 5min
4. Wash with 1ml of PBS1X twice
5. Ethanol Fixation: Resuspend the cells in 500µl of PBS1X, while vortexing the cells add 1.2ml ethanol 70% very slowly. Incubate at -20^{o}C for 3h or O/N. Cells can be stored in fixative at 4^{o}C for several days, for longer storage keep them at -20.
6. Spin the cells at 2000rpm for 10 min. At this step be careful when decanting the supernatant.
7. Wash with 1ml PBS 1X twice, spin at 2000rpm for 5min

8. Resuspend cells in 1ml of PBS containing 0.25% Triton X-100, incubate on ice for 15min.
9. Spin the cells at 2000rpm for 5min, wash once with 1ml of PBS 1X and spin
10. Resuspend the pellet in 100µl of anti-pH3 antibody dilution (1/1000). Dilute the antibody in PBS 1X+ 1%BSA
11. Incubate cells for 90min at room temperature (or O/N at 4C).
12. Add 1ml of PBS 1X + 1%BSA and spin the cells at 2000rpm for 5min.
13. Resuspend the pellet in 100µl of of anti-rabbit Alexa 488 dilution (1/250). Dilute the antibody in PBS 1X+ 1%BSA
14. Incubate cells for 30min at room temperature in the dark
15. Add 1ml of PBS 1X and spin at 2000rpm for 5min
16. Resuspend cells in 100 µl of PBS 1X containing RNAse A at 250µg/ml. Incubate cells for at least 30 min at 37C in the dark.
17. Add 100 µl of PBS 1X containing propidium iodide at 25µg/ml.

(can be stored at 4°C and re-analysed 24hr later if required.)

Reagents

70% ethanol **in PBS**
PBS 1X + 0.25% Triton X100
PBS 1X + 1% BSA (high-grade, A0846, Applichem)
Propidium Iodide 1mg/ml (Sigma)
RNAseA 100mg/ml (Sigma)
Phospho-Histone H3 (Ser10) (D2C8) XP® Rabbit mAb #3377 (Cell Signaling Technologies)
Anti-rabbit Alexa 488 secondary antibody (Life Technologies)

References

Protocol kindly provided by Elisabeth Zielonka/Hentze Group.