PROTOCOL CELL CYCLE 1 - STAINING

Fixed Cell Staining Protocols

DYE	LASER EXCITATION (nm)	EMISSION (nm) & COMMON FILTER	SELECTIVITY	SEQUENCE USED
DAPI	355 (UV) or 405 (Violet)	456 → 450/50	DNA	A/T
Propidium Iodide (PI)	561 (YG) or 488 (Blue)	617 → 610/20	DNA & RNA	A/T
7 AAD	561 (YG) or 488 (Blue)	647 → 670/30	DNA	G/C
Hoechst 33342	355 (UV) or 405 (Violet)	497 → 450/50	DNA	A/T
DRAQ5	640 (Red)	681 → 695/40	DNA & RNA	A/T

Example Protocol : PI Cell Cycle (For other protocols click here.)

1. Harvest cells and wash in PBS (with 2% FCS). Check by counting that the concentration is ~0.5 to 1 million cells/ml
2. Fix pelleted cells in ice-cold 70% ethanol by adding *dropwise* with a Pasteur pipette on a vortex. Leave cells at 4°C from 30 mins to a week.
3. Pellet cells at approximately 500 g for 5 mins. Wash **TWICE** in PBS.
4. Add 50 µl RNAse (final concentration of 100 µg/ml) and incubate at RT or 37°C for 15 mins.
5. Add 200 µl of PI to a final concentration of 50 µg/ml. Leave at RT or 37°C for at least 30 minutes.

IMPORTANT

- The concentration of the dyes in the protocol works for this number of cells. For different cell numbers titrate the DNA dye.
- Ethanol is used for fixation – do not use aldehydes of any kind
- Washing the cells twice ensures that ethanol is removed, any excess ethanol could affect the staining.
- Cell cycle profile can improve after 24 hours staining at 4°C.