P. Olivier — 1/2000

BrDU and PI staining for cell cycle analysis

Solutions:

0.08% w/v Pepsin:	Dissolve 0.4 g pepsin in 500 mL of 0.1M HCl (496 ml dd H20 + 4.1 mL conc. HCl), filter, store 4°C.
2M HCl:	For 417 mL ddH20 plus 83 mL conc. HCl. Filter, store RT.
0.1M Na Borate:	For 500 mL, 19.07 g Na borate, filter, store RT.
IFA:	10mM HEPES, pH 7.4; 150mM NaCl; 4% fetal bovine serum; 0.1% sodium azide. Filter, store RT.
IFA/Tween 20:	Add 0.5% Tween 20 to IFA.

Vortexing pellets while adding solutions is important.

Fixation of cells:

1. Trypsinize cells. Resuspend in PBS containing 5% serum (filtered to remove serum precipitate). Break up clumps by pipetting and spin down cells in 15 mL conical tubes.

2. Aspirate supe and vortex pellet. While vortexing, add 1.5 mL of cold PBS. While vortexing, slowly add 3 mL of cold 95% ethanol in a steady stream. Continue to vortex until thoroughly mixed. At this point samples may be stored at 4ºC for several days or longer.

Staining procedure:

Spins are done in a clinical tabletop centrifuge 5' at top speed, approx 1000 X g

1. Spin down cells, aspirate supe and vortex pellet.

2. While vortexing, add 3 mL of 0.08% pepsin. Incubate at 37°C for 20 min with occasional mixing.

3. Spin down nuclei (expect a very small pellet), aspirate supe and vortex pellet. While vortexing add 1.5 mL of 2M HCl. Incubate 20 min at 37°C with occasional mixing.

4. While vortexing add 3 mL 0.1M Na-borate. Spin down nuclei.

5. Aspirate supe and vortex pellet. While vortexing add 2 mL IFA/Tween 20.

6. Spin down nuclei, aspirate supernatant and vortex pellet. Add 75 µL of 1:5 dilution in IFA of anti-BrdU-FITC and incubate on ice in the dark for 30 min.

7. Add 2 mL IFA/Tween 20 while vortexing. Spin down nuclei, aspirate supe and vortex pellet. Resuspend in 0.25 mL IFA.

8. Add 0.25 mL of 100 µg/mL propidium iodide (in PBS) and incubate on ice in the dark for 60 min. Samples can be stored overnight (even a day or so is OK).

9. Run on FACScan.