**BrDU/PI Analysis**

Label cells in culture with 20 µM BrDU (20 mM stock in DMSO) for 20 to 30 minutes.

Harvest cells (at least 1 x 10⁶ stain) and fix with 1-2 ml 70% cold ethanol, suspending the cells first in PBS and then adding ethanol to 70% (Can store such cells at −20°C in the dark indefinitely).

Spin down cells and resuspend in 1 ml of 2.5M HCl/0.5% Triton-X 100 (make fresh). Incubate at RT for 25 minutes. (Conc. HCL = 12N)

Add 2.5 ml wash buffer (PBS + 0.5% Tween-20), spin down at 2,000 rpm, and wash two more times.

Add anti-BrDU-FITC in 100 ml (wash buffer + 1% BSA or 1.5% milk powder)/

Wash 1 X and resuspend in 1 ml PBS. Add 10-20 ul of 1 mg/ml propidium iodide and 100 ug/ml RNase A (10 ul of 10 mg/ml). Incubate at least 30 minutes at RT.