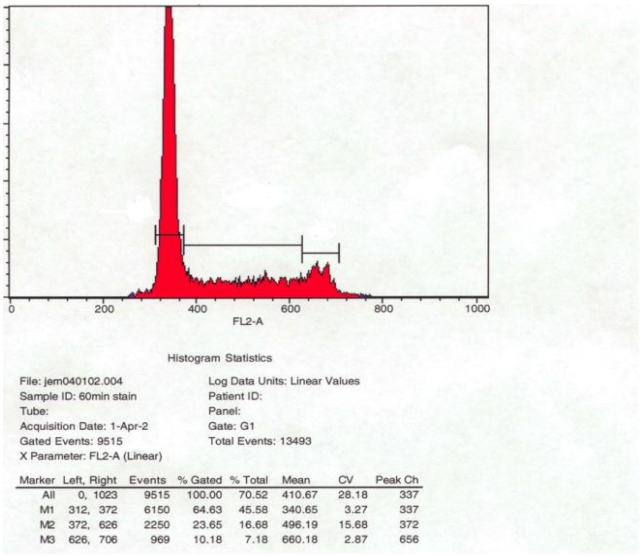
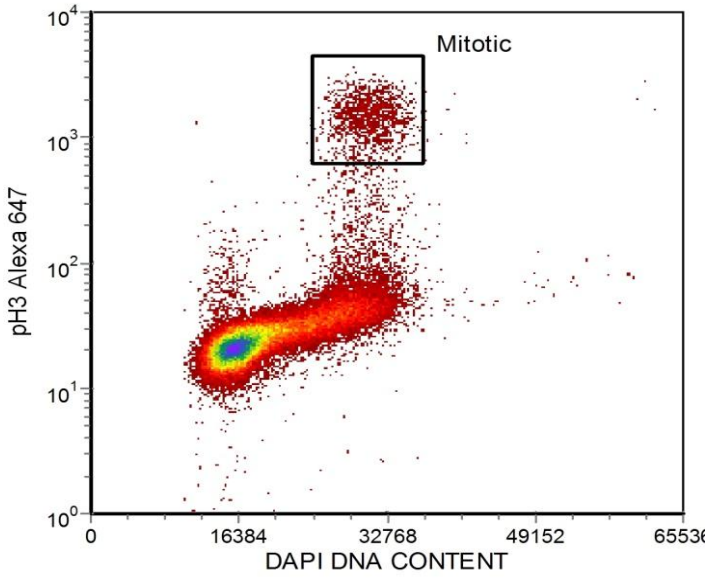
# Cyclin Protocol




**DAPI or PI**

**Procedure:**

1) Wash 1x$10^6$ cells once with 2ml PBS.
2) Resuspend in 100µl PBS.
3) While vortexing, add 100µl of a 1% Formaldahyde solution and incubate in a 37°C water bath for 10min.
4) Add 800µl ice cold absolute Methanol drop-wise and incubate at 4°C for 30min. (longer is fine; can be held up to several weeks at -20°C).
5) Wash 2X in PBS, Spin at 800g for 5min. (must spin harder when in Methanol to minimize cell loss).
6) Resuspend in 50µl PBS/2%BSA and add antibody, incubate at 4°C for 30min.
7) Wash 2X
8) Resuspend in 500µl DAPI solution or 500µl PI solution (not both). **NOTE:** If PI solution is used, incubate at 37°C for 20 minutes (necessary for RNase A).
9) Incubate for 1hr in fridge and analyze.




**Solutions to make:**

-**1% Formaldahyde solution:** 62.5µl of 16% PFH and 937.5µl PBS

-**10µg/ml DAPI solution:** 10ml PBS and 20µl of 5mg/ml DAPI

-**Propidium Iodide (PI) staining solution:** To 10ml of PBS, add 500ul of a 1mg/ml solution of PI, 2mg of RNase A, and 100ul of 10% Triton-X solution (final concentrations: 50µg/ml PI, 0.1% Triton-X).