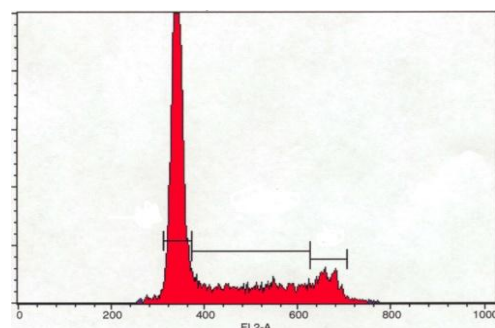


Cyclin Protocol

DAPI or PI

Procedure:

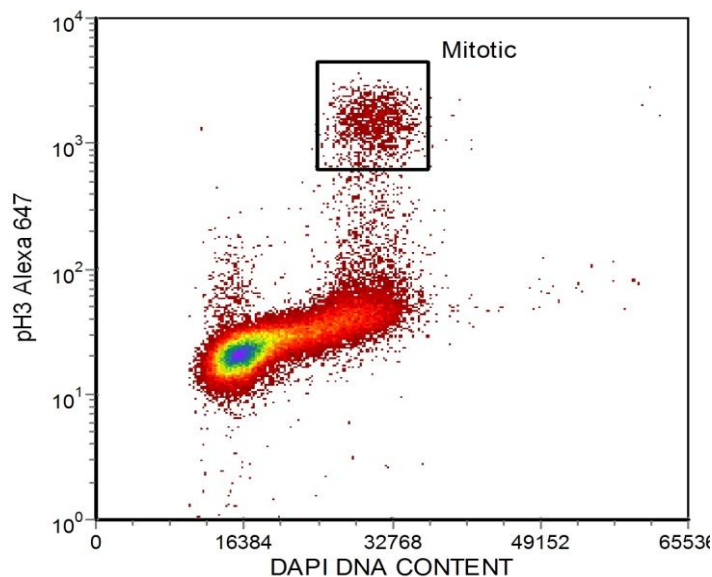
- 1) Wash 1×10^6 cells once with 2ml PBS.
- 2) Resuspend in 100 μ l PBS.
- 3) While vortexing, add 100 μ l of a 1% Formaldehyde solution and incubate in a 37°C water bath for 10min.
- 4) Add 800 μ l ice cold absolute Methanol drop-wise and incubate at 4°C for 30min. (longer is fine; can be held up to 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 50 μ l PBS/2%BSA and add antibody, incubate at 4°C for 30min.
- 7) Wash 2X
- 8) Resuspend in 500 μ l DAPI solution or 500 μ l 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.



Histogram Statistics

File: jem040102.004 Log Data Units: Linear Values
 Sample ID: 60min stain Patient ID:
 Tube: Panel:
 Acquisition Date: 1-Apr-2 Gate: G1
 Gated Events: 9515 Total Events: 13493
 X Parameter: FL2-A (Linear)

Marker	Left	Right	Events	% Gated	% Total	Mean	CV	Peak Ch
All	0	1023	9515	100.00	70.52	410.67	28.18	337
M1	312	372	6150	64.63	45.58	340.65	3.27	337
M2	372	626	2250	23.65	16.68	496.19	15.68	372
M3	626	706	969	10.18	7.18	660.18	2.87	656



Solutions to make:

-1% Formaldehyde solution: 62.5 μ l of 16% PFH and 937.5 μ l PBS

-10 μ g/ml DAPI solution: 10ml PBS and 20 μ l of 5mg/ml DAPI

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

