Propidium idodide staining for cell cycle/apoptosis analysis

1. Make 70% ethanol and Staining Buffer (0.1% Triton-X100, 20ug/mL Propidium Iodide, and 0.2mg/mL of DNAse free RNaseA). These solutions should be ice-cold before one starts.
2. Harvest the cells.
3. Wash the cells, now in suspension, once with PBS. Use 15 mL conicals if possible. Wash with as much PBS you can add to the centrifuge tube. Wash for 5 minutes at 450xg for lymphocytes. Remove most of PBS after spinning by aspiration.
4. Resuspend the pellet to **a single cell suspension as much as possible.**
5. Add 2 mL of 70% ethanol (0.5 mL if microfuge tubes are used) as quickly as you can to the cells, using a vortex (speed= medium to low) to resuspend the cells as the ethanol is added
6. Spin cells for 10 minutes at 450xg
7. Remove the ethanol and resuspend the pellet.
8. Add 2mL (1 mL if microfuge tubes are used) of the Staining Buffer
9. Incubate in the dark at room temperature for at least an hour or overnight at 4 degC
10. Perform flow cytometry analysis for cell cycle. Flow rate should be around 100 events per second. Spin cells for 10 minutes at 450xg to enrich if the suspension is too dilute. A minimum of 50,000 collected events gives a smooth curve.