- 2. Fix in 1ml cold 70% ethanol. Add drop wise to cell pellet while vortexing. This should ensure fixation of all cells and minimise clumping.
- 3. Fix for at least 30 minutes on ice. Specimens can be left at this stage for several weeks (make sure you seal the tubes for long term storage).
- 4. Pellet cells at higher speed for 5 minutes; decant the supernatant being careful not to lose the pellet. Note that ethanol-fixed cells require higher centrifugal speeds to pellet compared to unfixed cells since they become more buoyant upon fixation.
- 5. Wash once with PBS + 0.1% Triton X solution.
- 6. Wash once with PBS + 0.1% Triton X + 3% BSA.
- 7. Pour off supernatant and add directly to the pellet 2µl of rabbit Anti-Phospho Histone 3 antibody, mix well and incubate at room temperature for 45 minutes.
- 8. Wash twice with PBS + 0.1% Triton X solution.