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.

Flow Cytometry Core Facility, Camelia Botnar Laboratories, Room P3.016
UCL Institute of Child Health. 30 Guilford Street, London WC1N 1EH
Tel 020 7405 9200 ext 0198