**Flow Cytometry Apoptosis Protocol**

1. Dilute apoptosis binding buffer from 10x to 1x

1 mL per sample

1. Harvest cells as normal => KEEP ALL MEDIA IN WELL AND PBS USED TO WASH CELLS
2. Centrifuge at 4 degrees, 2000 rpm for 5 minutes
3. Pour off and resuspend in cold PBS
4. Centrifuge as above
5. Pour off and resuspend in cold PBS
6. Centrifuge as above
7. Resuspend in 100 uL binding buffer and transfer to FACS tubes
8. Add 3 uL of Annexin V conjugate
9. Mix gently by flicking, incubate in dark for 15 minutes
10. Add 400 uL of binding buffer and 5 uL of 50 ug/mL PI and analyse within an hour