



## **Equipment**

1. **Centrifuge.**
2. **Pipettes.** You will need two: one in the range of 10-100 $\mu$ l, and another ranging from 100-1000 $\mu$ l.
3. **12x75 mm polystyrene/polypropylene tubes.** Depending on which machine you wish to use (LSR II prefers polystyrene while the CyAn prefers polypropylene).
4. **Ice bucket with cover.** Generally, cells are more stable and tolerate stress better when they're cold. The cover keeps samples in the dark and prevents light from bleaching the fluorochromes.
5. **Flow cytometer.** We have a variety of machines at your disposition including a BD LSR II, BD FACSAria and a Beckman Coulter CyAn. Each machine has different capabilities, strengths and weakness so be sure to check with us which machine is suited for your needs.

## **Procedure**

1. Harvest your cells and, if you can, adjust the number of cells to roughly 1 million per sample.
2. Dilute the Annexin V conjugate 1 in 100 in Annexin V binding buffer. (if staining more than one sample, prepare a batch solution for all samples and mix well).
3. Resuspend each pellet in 500 $\mu$ l Annexin V conjugate/binding buffer mixture.
4. Keep the samples at RT in the dark for around 20 minutes (30 or more minutes on ice), cover the ice bucket.
- 5.