

Equipment

1. **Centrifuge.**
2. **Pipettes.** You will need two: one in the range of 10-100 μ l, and another ranging from 100-1000 μ 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 μ 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.