

Annexin V Staining:

- Harvest cell by centrifugation (Usually 1×10^6 cells per sample).
- Gently resuspend pellet and wash once with 1X Annexin HEPES buffer + 2% FBS.
- Gently resuspend pellet in 1X Annexin HEPES buffer + 2% FBS at 20×10^6 cells/ml (If you do not have enough cells for 1×10^6 cells per sample, resuspend at $50 \mu\text{l}$ per sample with at least 100,000 cells per sample).
- Add appropriate amounts of Annexin to 12 x 75 mm polystyrene test tubes.
- If performing additional surface staining, commercial antibodies are stored in buffers without high calcium. Annexin V should be added to cells prior to combining cells with additional antibodies. SEE BELOW.
- Add $50 \mu\text{l}$ of the cell suspension above (usually 1×10^6 cells per sample) and gently vortex.
- Incubate on ice for 20 minutes in reduced light.
- Wash 1 time with ice cold Annexin HEPES buffer + 2% FBS (3 ml) at 4°C .
- Repeat wash for tubes containing Annexin FITC including the "Auto" sample - fix the remaining samples during this wash.
- Gently resuspend pellet and add cold 4% paraformaldehyde in 1X PBS ($400 - 600 \mu\text{l}$ per sample) to fix.
- Store in the dark at 4°C until run on flow cytometer.

Notes:

1-Annexin V staining must be done on LIVE CELLS!! Fixed cells are always positive.

Therefore, if the wash is not sufficient, cells will all be annexin positive.

2-Annexin V staining requires high Calcium; therefore you MUST use the annexin buffer.

Resuspend cells in the annexin buffer plus annexin V and then add additional antibodies.