

## Apoptosis Measurement using AnnexinV and 7-AAD

AnnexinV is an antibody that binds to the phospholipid phosphatidylserine when it translocates across the lipid bilayer and is a sign of a cell undergoing apoptosis.

7-AAD (7-Aminoactinomycin D, FL-3) is an exclusion dye that stains cells that are already dead.

Both stains and binding buffer (10X) come as a kit from BD Biosciences (Cat. No. 559763).

1. After culture wash cells twice in ice cold PBS.
2. Add AnnexinV in 1x Binding Buffer at an amount that is required for two tests (usually 5 $\mu$ L for normal apoptosis detection).
3. Incubate cells for 15 minutes at room temperature in the dark.
4. Add 7-AAD the same volumes just before samples are analyzed.
  - a. Alternatively, Propidium Iodide (PI) can be used in place of 7-AAD.