-we ask for 3 hours of your day to complete the session.
-for training, you should bring:

-controls:
-negative control:
tube #1: consisting usually of cells with no treatment and no staining (i.e., no Propidium Iodide (PI), no Annexin-V, etc.)

-positive controls:
HINT: A good positive control for apoptosis: Heat shock the cells. Incubate 1 minute @ 56°C followed by inculcation @ 37°C dependent on cell type. Usually 1 hour after shock is sufficient to induce 50% apoptosis for most cell lines.
-for apoptotic studies, we will need:
tube#2: cells + PI, no treatment
tube#3: cells + Annexin-V, no treatment
tube#4: cells + PI + Annexin-V no treatment
tube#5: cells + Annexin-V + treatment
tube#6: cells + PI + treatment
tube#7: cells + PI + Annexin-V + treatment

Annexin-V is considered an early apoptotic indentifier, which binds to phosphatidylserine (PS) residues that have been translocated from the inner cytoplasmic membrane and externalised during apoptosis.

There are many commercial sources of Annexin which can be conjugated to a number of fluorochromes. We have used annexins labelled with FITC, Alexa488, PE, APC and Alexa647. The choice of fluorochrome will be influenced by any other fluorochromes that you are measuring. If using GFP-containing cells, the best combination of dyes for live/dead/apoptotic analysis is Annexin-Cy3/TO-PRO-3 or Annexin-Alexa647/PI.

We have had good experience with Annexin-V-PE and 7-AAD.

The above protocol will get us to the point that we can identify areas of the experiment that must be tweaked (i.e., changes in number of cells/concentration of reagents, etc.). We have seen historically that many apoptosis detection kits use Jurkat cells for quality control, and many times the protocol included must be altered or modified for our own cell-types. We want not only a reliable protocol, but also one that is repeatable and therefore useful to us in the future.

Let us know if you have any questions.