**Protocol for Apoptosis**

Preparation Annexin V Binding Buffer- Dilute to 1x Annexin V (withdraw 5ml 10x Annexin V to 50ml UltraPure H2O)

1. Withdraw sample from well to eppendorf (use 1.5mL Eppendorf)

\*No of cell depends on the apoptosis. If need few cycles to reach apoptosis, then have to decide. Use min 10k cell.

\* Must have negative control (stained sample)

Positive control and unstained sample (total 3 extra tubes)

1. Spin down the sample 1200-1500rpm 5 mins. Withdraw supernatant.
2. **Wash cell with sterile 1xHBSS solution** (one or twice).
3. Resuspend the samples (3-5 secs) and centrifuge at 1200-1500rpm for 5 mins.
4. Withdraw supernatant.
5. Take **FITC Annexin V** from 4C fridge (small long tube) and PI. Prepare FITC Annexin V mastermix (1:20@50) – Add 5uL of FITC Annexin V to each 100uL of sample cell solution in the eppendorf.
   * Eg (1:50) if 5 tubes prepare 6 tubes amounts
   * 100uL x 6 Annexin V buffer + 2uL x6 FITC Annexin V + 2uL x 6 PI@ 7AAD (Mastermix)
6. Add in PI@**7AAD** into the mastermix (Ratio 1: 20@50)
7. Prepare 1 tubes with sample for unstained, stain with FITC Annexin V only, stained with PI only. Add in solution separately. (Eg. 100uL Binding Buffer+ 2uL FITC Annexin V@ 2uL PI)
8. Gently vortex the samples.
9. Incubate at RT 15 mins in dark @ 4C for 30 mins.
10. Label the **flow tubes** accordingly.
11. Add in 400-500uL Annexin V binding buffer (Solution only) (into each flow tube) (\*Amount of binding buffer to add as long as is same for each tube).
12. Add in 100uL of sample into each tube (meaning 400uL +100uL).
13. Run flow cytometry.

**Protocol for Flowcytometry Machine**

1. Switch on Computer.
2. Choose the CXP icon.
3. Create a new file.
4. Rearrange the SS (y-axis) and FS (x-axis).
5. Click polyglonal icon on the top bar to create FL1 & FL3@4.
6. Click another icon to open the 3rd graph… (Follow the video from Nelson)
7. To top up sheath fluid, remember to **click icon *Zzz*** on the comp before open the lid of the machine.

**FL1- FITC**

**FL2- PE**

**FL3- PI**

**FL4- APC (need laser)**

**7AAD (Apoptosis)**

CD11b- Differentiation marker

Acc: CHM

P/W: himm2021