Mitochondrial Membrane Potential, Annexin V, and DAPI

MitoTracker Red (CMX-Ros) Treatment with Annexin V plus DAPI

For each time-point, dye controls are needed. The "no dye" control could be from a flask of untreated cells not specifically used in the experiment. It should be processed in parallel with the experimental flasks, but no dyes added. A MTR control can be made simply by pooling a small fraction of each sample prior to the addition of Annexin V dye.

1. At the desired time-point, add 5ul of MTR stock to each flask (final concentration o100nm),being sure to swirl sufficiently. Alternatively, the stock can be diluted with PBS (e.g. 1:10), and a proportionaltely larger volume of dye added.

2. Incubate at 37degrees C for 30 minutes.

3. Remove the media from the T-25 flasks and place in labeled conical vials.

4. Wash the remainin cells twice with 1ml PBS.

5. Add.75 ml Tryposin and incubate for about 5 minutes at 37 degrees.

6. Collect the cells with approximateely 2 ml of the media saved proviously and combine all in the appropriated tubes.

7. Spin down at 1200 RPM for 5 minutes

8. Remove the supernattant and wash pellet in 1ml of cold PBS.

9. Spin down at 1200RPM for 5 minutes.

10. Remove the supernattant and re-suspend in 400ul of Binding buffer or ~1x 106 cell/ml.

11. Transfer 100-200ul of cell suspension in to flow tubes.

12. Add 5 ul of Annexin V- (FITC, Alexa 488) dye to each sample. Add 100ng DAPI to each sample.

13. Process via flow collecting with APC for MitoTracker, DAPI for DAPI, and FITC for Annexin.