Moxi Flow Apoptosis (PE-Annexin V) and Viability (PI) - Staining Protocol

Reagents:

- Annexin V Binding Buffer (BioLegend, cat#422201)
- Cell Staining Buffer (BioLegend, cat#420201)
- PE-Annexin V conjugate (BioLegend, cat#640907)
- Orflo Flow Reagent (Orflo Technologies, MXA080)
- Moxi Cyte Viability Kit (Orflo Technologies, MXA055)

Protocol

1. Induce apoptosis in cells by desired method (e.g. 20µM Camptothecin treated, 6+ hours, 37°C) Jurkat cells. Include a control sample of untreated cells.
2. Isolate cells to a single-cell suspension. (If necessary use a protease and/or pipette trituration to break apart the clusters)
3. Wash cells twice in Cell Staining Buffer (2.5mL volume, 300 x *g*, 5 minutes).
4. Re-suspend pellet to 1 x 10^6 cells/ml in Annexin V Binding Buffer (verify counts with the Moxi Z or Moxi Flow instruments).
5. For each sample, prepare two microcentrifuge tubes. *Note: the tubes can be processed concurrently to reduce overall processing time.*
   - ***Tube 1 (PE- Annexin V measurement):***
     a. Add 100µl of cells
     b. Add 10 µl of PE-Annexin V conjugate*. Notes: Required PE-Annexin V concentration may vary by PE-Annexin manufacturer. We recommend 0.3 – 0.6µg/ml final PE – Annexin V concentration.*
     c. Gently vortex the cells and incubate for 15 minutes at room temperature (25°C), protected from light.
     d. Wash cells twice (1mL volume, 300 x *g*, 5 minutes). with *Annexin V Binding Buffer* (critical, using a low Ca^{2+} or Ca^{2+}-free buffer will disrupt Annexin binding)
     e. Re-suspend pellet in 400µL of *Annexin V Binding Buffer*
     f. Add 8 uL of Moxi Cyte Flow Reagent. Vortex to mix.
     g. Run on Moxi Flow using the "*Apoptosis (Annexin V-PE)*" app within 2 hours of staining, protect from light.
   - ***Tube 2 (PI/Viability measurement):***
     a. Add 60µL of cell suspension to 240µL of Moxi Cyte Viability Kit (5x dilution)
     b. Gently vortex the cells and incubate for 5 minutes at room temperature (25°C), protected from light.
     c. Run on Moxi Flow using the "*Viability Count (PI)*" app within 2 hours of staining, protect from light.
6. As all membrane-permeable (dead) cells will stain positive for PE-Annexin V, the early-stage apoptosis percentage can be calculated as the PE-Annexin V positive percentage (from the first test) minus the PI positive percentage (from the second test).