Moxi GO II – Apoptosis Kit (MXA701)
Apoptosis Monitoring with FITC-Annexin V and Propidium Iodide (PI)

Instrument/Cassettes:

- Moxi GO II Next Generation Flow Cytometer (Gemini Bio-Products, Cat # MXG102)
- Type S+ Cassettes (Gemini Bio-Products, Cat# MXC030/MXC032)

Reagents/Components:

- Moxi Cyte Apoptosis Kit (Gemini Bio-Products, Cat# MXA701). Containing:
  - Reagent # 1: FITC – Annexin V
  - Reagent # 2: Propidium Iodide
  - Reagent # 3: Annexin V – Binding Buffer

Protocol:

Notes:

- *For comparison purposes, it can be useful to generate a positive control by inducing apoptosis with a pharmacological agent (e.g. 30µM Camptothecin treated, 4+ hours, 37°C for Jurkat cells).*
- *Process a sample of healthy, untreated, cells for use as a negative control.*

1. Isolate cells to a single-cell suspension. *Note: If necessary, use a protease (e.g. Accutase, GemBio Cat #400-158) and/or pipette trituration to break apart the clusters.*
2. *(Optional) For improved staining results, particularly with adherent cells, pre-Wash cells 1x (300xg, 5min) with PBS or equivalent.*
3. Pellet Cells (300xg, 5min) Re-suspend pellet to ~1 x 10^6 cells/ml in **Reagent #3: Annexin V *Binding Buffer***.
4. Aliquot 100 µl of cells to a microcentrifuge tube (~1x10^5 total cells). Mix well before aliquoting.
5. Add 5µL of ***Reagent #1: FITC - Annexin V conjugate.*** *Note: While 5µL should work for most cell samples, it may be necessary to titrate the Reagent #2 volume to optimize the signal.*
6. Add 5µl of ***Reagent #2: Propidium Iodide (PI)***
7. Gently vortex (3-4 setting) the cells and incubate for 15 minutes at room temperature (25°C), protected from light.
8. Add 390µL of ***Reagent #3: Annexin V Binding Buffer*** to all tubes.
9. Run on Moxi GO II using the "*Apoptosis (Annexin V - FITC & PI)*" app within 15 minutes of staining. Protect from light. *Notes:*
   a. *This kit was designed to be used with the 646nm/LP filter installed in the back (PMT2) slot of the Moxi GO II. Using the 561nm/LP filter will require compensating for spillover (FITC into PMT2) and possibly lowering the PI concentration so that it is not too bright.*
   b. *Adjust size gates to define the cell population.*
   c. *Touch "Next" view PMT vs PMT display of the FITC Annexin (PMT1) vs. PI (PMT2) fluorescence. Adjust the gate markers to identify the relevant cell sub-populations/*
   d. *Once gated, touch "Summary" for a bar chart/table summary view of the data.*