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

Instrument/Cassettes:

- Orflo Moxi GO II Next Generation Flow Cytometer (Orflo Cat #MXG102)
- Compatible Orflo Cassette (any of the following would work):
  - Type CST (Orflo Cat# MXC040)
  - MF-S (Orflo Cat# MXC020)
  - MF-S+ (MXC030)

Reagents/Components:

- FITC-Annexin V conjugate (e.g. BioLegend, cat#6409075)
- Annexin V Binding Buffer (e.g. BioLegend, cat#422201)
- Propidium Iodide (PI) staining solution (100µg/ml in PBS) – This can be made by diluting Thermo P3566 (1mg/ml PI) 10x with Purified water (e.g. Sigma W4502)
- *Optional/Recommended: Orflo Flow Reagent (Orflo Cat #MXA080)*

Protocol:

Notes:

- *For comparison and compensation 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. (If necessary use a protease and/or pipette trituration to break apart the clusters)
2. Wash cells twice in PBS (2.5mL volume, 300 x *g*, 5 minutes).
3. Re-suspend pellet to 1 x 10^6 cells/ml in Annexin V Binding Buffer (verify counts with the Moxi GO II instrument).
4. Aliquot 100 µl of cells per tube (~1x10^5 total cells). Mix well before aliquoting. For compensation, prepare three tubes:
   a. PI only sample
   b. FITC - Annexin V only sample
   c. FITC - Annexin V and PI Sample
5. Add 1-5µL of of manufacturer recommended test volume of FITC - Annexin V conjugate (i.e. 2µL for BioLegend Annexin V listed above). *Note: Titration of the Annexin dose might be necessary. Recommended Mfg. volumes are typically 5µL.*
6. Gently vortex (3-4 setting) the cells and incubate for 15 minutes at room temperature (25°C), protected from light.
7. *Optional: To lower the background (improve signal to noise ratios) for bright samples, a 1-2x wash (300xg, 5min) with binding buffer will remove the excess FITC – Annexin conjugate.*
8. Add 300µL of Annexin V Binding Buffer to all tubes.
9. Add 2.5 µl of 100µg/ml Propidium Iodide (PI) to appropriate tubes (target final concentration of 1µg/ml PI). Incubate those tubes for 5 additional minutes.
10. *Optional: Add 8µL of Orflo Flow Reagent to sample (20µL flow reagent / ml of sample)*
11. Run on Moxi GO II using the "*Apoptosis (Annexin V - FITC & PI)*" app within 15 minutes of staining, protect from light.

a. A

djust size gates to define the cell population.

b. Touch "X/Y" to select a PMT vs PMT display of the FITC Annexin (PMT1) vs. PI (PMT2) fluorescence.

c. Adjust for spillover as appropriate using the "Comp" button functionality.