

## Moxi GO II - Apoptosis (FITC-Annexin V) and Viability (PI) - Staining

### Instrument/Cassettes:

- Orflo Moxi GO II Next Generation Flow Cytometer ([Orflo Cat #MXG102](#))
- Orflo Type S+ Cassettes (Orflo Cat# MXC030/MXC032)

### Reagents/Components:

- FITC-Annexin V conjugate (e.g. [BioLegend, cat#640905](#))
- Annexin V Binding Buffer (e.g. [BioLegend, cat#422201](#))
- Propidium Iodide (PI) staining solution (1mg/ml in PBS) (e.g. [Thermo P3566 \(1mg/ml PI\)](#))
- *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. 30 $\mu$ M 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. Pellet cells (300 x g, 5 minutes).
  3. Re-suspend pellet to 1 x 10<sup>6</sup> cells/ml in Annexin V Binding Buffer (verify counts with the Moxi GO II instrument).
  4. Aliquot 100  $\mu$ l of cells to a microcentrifuge tube (~1x10<sup>5</sup> total cells). Mix well before aliquoting.
  5. Add 1-5 $\mu$ L of of manufacturer recommended test volume of FITC - Annexin V conjugate (i.e. 5 $\mu$ L for BioLegend Annexin V listed above). *Note: Titration of the Annexin dose might be necessary. Recommended Mfg. volumes are typically 5 $\mu$ L.*
  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 300 $\mu$ L of Annexin V Binding Buffer to all tubes.
  9. Add 2 $\mu$ l of 1mg/ml Propidium Iodide (PI) (target final concentration of 5 $\mu$ g/ml PI). Incubate for 5 additional minutes.
  10. *Optional: Add 8 $\mu$ L of Orflo Flow Reagent to sample (20 $\mu$ L 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. To avoid the need to compensate for the FITC spillover into the PI channel, make sure the 646nm/LP filter is installed in the back (PMT2) slot
    - b. Adjust size gates to define the cell population.
    - c. Touch “X/Y” to select a PMT vs PMT display of the FITC Annexin (PMT1) vs. PI (PMT2) fluorescence.