Viability Staining (PI) Protocol

Overview:

This viability protocol is based on the simple principle of cell membrane exclusion. Briefly, as cells die, ruptures/holes form in the membrane that allow for the entry of larger molecules that ordinarily would be blocked. In this manner, viability dyes such such as propidium iodide (PI) are excluded from the interior of "live" cells and allowed to enter "dead" cells. The result is that the dead cells accumulate the dyes and brightly fluoresce (PI). PI is particularly attactive as it exhibits a ~40x increase in fluorescence upon binding with DNA (after it enters the cell) resulting a large signal increase, relative to the shading level change associated with Trypan Blue.

Reagents required:

- Dilution Buffer (e.g. PBS)
- Propidium Iodide (PI) stock solution - 1mg/ml (e.g., ThermoFisher Cat#P3566)
- *Optional/Recommended:* Orflo Flow Reagent (Orflo Cat #MXA080)

Viability (PI) Staining

1. Dilute cells to 1 - 5 x 10^5 cells/ml (recommended range) using Dilution Buffer
   Note: As a rule of thumb, for samples with unknown concentrations, start with a 10x dilution.
2. Add 2µL of (1mg/ml) PI stock solution for every 1mL of cell solution (final PI concentration of 2µg/mL).
3. Incubate for 5 minutes in the dark at room temperature (25°C).
4. *Optional/Recommended:* Add 20µL of Orflo Flow Reagent per ml of cell sample.
5. Analyze with the Moxi Flow or Moxi GO using the "*Viability Count (PI)*" app within 10 minutes.
   Note: For the Moxi GO, make sure the 561nm/LP filter is installed.