Moxi GO II – Measuring Cell Health/Vitality with Calcein-AM and Propidium Iodide (PI)

Overview:

Propidium (PI) is a cell membrane-impermeant, DNA-intercalcating dye that increases fluorescence over 50x upon binding to DNA. For healthy cells, the dye is excluded and no fluorescence signal is associated with the cell event. However, as cells lose membrane integrity through necrosis or apoptosis, the PI permeates the cell and a strong fluorescence signal is generated. In this way, PI is applied to measure cell "viability" through the dye-exclusion approach. However, the simple exclusion of a dye does not necessarily reflect the metabolic state of the cell which many researchers regards as a more critical metric of cell viability or "vitality." Towards this end, several dyes (e.g. calcein-AM) are available that are designed to measure the esterase enzyme activity, a family of enzymes believed to be common to all cells. To measure this activity, calcium sensitive dyes (e.g. Calcein) are conjugated to acetoxymethyl ester moieties (e.g. calcein-AM) that serve to simultaneously assist in the membrane permeability of the dyes as well as quench the dyes fluorescence. Once loaded, the constitutive activity of the esterase enzyme (in metabolically active cells) cleaves the AM functionality of the molecule causing it to fluoresce and slowing the rate of effusion from the cell. The result is a measureable cellular fluorescence that can be quantified and correlated to the metabolic state of cells. This protocol describes the implementation of this "Cell Health" or "Vitality" assay on the Orflo Moxi Go II systems.

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:

- Calcein-AM (Thermo Cat#C3100MP)
- Phosphate Buffered Saline (PBS, e.g. Thermo 10010023)
- Propidium Iodide (PI) staining solution (e.g. Thermo P3566 (1mg/ml PI)
- DMSO (any brand, e.g. Sigma #D8418)
- *Optional/Recommended: Orflo Flow Reagent (Orflo Cat #MXA080)*

Calcein-AM dye Preparation

- ***Calcein AM 2mM Stock*** - Create a 2mM stock solution of the AM dye. E.g. for Thermo Cat#C3100MP add 25.2µL of DMSO to the 50µg vial. Vortex to mix.
- ***Calcein-AM Working solution (10µM)*** – Create a working solution of Calcein-AM by diluting 2µL of the 2mM Calcein-AM stock into 398µL of DMSO.

Protocol:

1. Dilute cells with PBS to get sample into the optimal concentration range for the Moxi Flow. Final target concentration: ~1e5 – 5e5 cells/mL.
2. For each sample to be stained, add 500µL of diluted cells into a microcentrifuge tube. Note: It is useful to prepare a negative control by heat-killing a sample (60-70°C, 10min). For compensation, prepare three tubes:
    a. PI only sample with the heat killed cells
    b. Calcein-AM only sample with the Healthy/Live (control) cells
    c. Calcein-AM and PI Sample for target cells
3. Add 2.5µL of 10µM Calcein-AM Working Solution to the appropriate tubes (final concentration (0.05µM Calcein-AM). Please note that, for different cell lines, it may be necessary to perform a titration of the Calcein-AM concentration to determine the optimal staining concentration to prevent railing of the system (too bright) or to achieve sufficient signal (to dim).
4. Add 0.5µL of 1mg/ml PI to the appropriate tubes (final concentration 1µg/ml, 1.5µM)
5. Vortex gently, Incubate samples at 37°C for 15min in the dark. Longer incubation times can be applied for increased Calcein-AM signals. Note: If longer times are used, we would recommend adding the PI 5-10minutes prior to the end of the Calcein-AM incubation.
6. *Optional/Recommend:* Add 10µL Orflo Flow Reagent to each sample and inversion mix.
7. Run on Moxi GO II using the "*Cell Health (Calcein-AM & PI)*" app immediately:
    a. Adjust size gates to define the cell population.
    b. Touch "X/Y" to select a PMT vs PMT display of the Calcein-AM (PMT1) vs. PI (PMT2) fluorescence.
    c. Adjust for spillover as appropriate using the "Comp" button functionality.