Moxi GO II - Mitochondrial Membrane Potential TMRE Staining

Overview:

Tetramethylrhodamine, ethyl ester (TMRE) is a cell-permeant fluorescent dye that, due to it's cationic nature, is readily sequestered by active mitochondria. TMRE has a peak excitation of 549nm but can be excited by either the 488nm or 532nm laser. The peak emission is 574nm, requiring the use of the 561nm/LP filter on the Moxi GO II instruments.

Reagents/Components:

- Orflo Moxi GO II Next Generation Flow Cytometer (Orflo Cat #MXG102)
- MF-S+ Cassettes (Orflo Cat #MXC030)
- Tetramethylrhodamine ethyl ester perchlorate (e.g. Sigma Cat #87917)
- Cell Staining Buffer (BioLegend cat # 420201 or PBS with 0.5% BSA, 0.1% Azide). *Note: Stock PBS (any formulation, e.g. Gibco, Cat #10010023) can be used but might have slightly lower signal-to-noise ratios (higher background) for the assay.*
- DMSO (any brand, e.g. Sigma #D8418)
- *Optional/Recommended*: Orflo Flow Reagent (Orflo Cat #MXA080)

Mitochondria - TMRE Labeling Protocol

1. Make initial stock solutions of TMRE by sequential dilution of TMRE (Sigma Cat #87917) as follows:
    a. 10mM stock TMRE: 25mg dissolved TMRE in 4.85ml DMSO
    b. 10µM stock TMRE: 10µL of 10mM TMRE stock in 990µL DSMO
2. Dilute cells to a concentration of ~2e5-3e5 cells/ml with Cell Staining Buffer. *Notes:*
    a. *Cells can be labeled directly in in culture media if they are already at the correct concentration (or are under-concentration).*
    b. *Cells need to be in a single cell suspension for testing. Detachment with Accutase/Accumax is recommended with pipette trituration to break apart clusters*
3. Aliquot 500µL of cell suspensions (it is useful to generate an FCCP-treated (i.e. 100µM), Sigma Cat #C2920, sample as a negative control) into separate polypropylene micro-centrifuge tubes (Santa Cruz, Cat #sc-200271) – *Note: do not use polystyrene (PS) as TMRE can bind significantly to PS.*
4. Add 2.5µL of 10µM TMRE stock solution to each vial to achieve a 50nM final TMRE concentration and gently vortex to disperse. *NOTE: At high overly high concentrations, TMRE has a quenching effect. Consequently, optimal TMRE concentration is dependent on cell type and sample prep and can vary from 20nM –200nM. Initial titration tests should be performed for optimal concentrations. As an initial guess, start with 50nM TMRE.*
5. Incubate TMRE/Cell media at 37°C for 15-30 minutes in the dark.
6. Post-incubation, incubate cells for an additional 15min (RT/Dark).
7. *Optional/Recommended: Add 20µL Orflo Flow Reagent per ml of cells and inversion mix sample.*
8. Immediately run the samples on the Moxi GO II system using the "Open Flow Cytometry" assay with the "Medium" gain setting.