

## Moxi GO II - Mitochondrial Membrane Potential TMRE Staining

### Overview:

Tetramethylrhodamine, ethyl ester (TMRE) is a cell-permeant fluorescent dye that, due to its 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. 10 $\mu$ M stock TMRE: 10 $\mu$ L of 10mM TMRE stock in 990 $\mu$ L DMSO
2. Dilute cells to a concentration of  $\sim 2 \times 10^5$  -  $3 \times 10^5$  cells/ml with Cell Staining Buffer. *Notes:*
  - a. *Cells can be labeled directly 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 500 $\mu$ L of cell suspensions (it is useful to generate an FCCP-treated (i.e. 100 $\mu$ M), Sigma Cat #C2920, sample as a negative control) into separate polypropylene microcentrifuge tubes (Santa Cruz, Cat #sc-200271) – *Note: do not use polystyrene (PS) as TMRE can bind significantly to PS.*
4. Add 2.5 $\mu$ L of 10 $\mu$ M 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 20 $\mu$ L 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.