

## Quantify Mitochondrial Membrane Potential

### Application:

Assess and monitor the mitochondrial membrane potential (MMP) of cells embedded in three-dimensional (3D) tissue constructs. The potentiometric dye, tetramethylrhodamine ethyl ester (TMRE), is used to label cellular mitochondria, and TMRE fluorescence is quantified using a plate reader.

### Introduction:

A great majority, ~80-90%, of cellular energy production occurs through oxidative phosphorylation. In this process, proton pumps establish an electrochemical gradient across the inner mitochondrial membrane, i.e., the MMP. The energy stored in the MMP is then used to drive the synthesis of ATP.

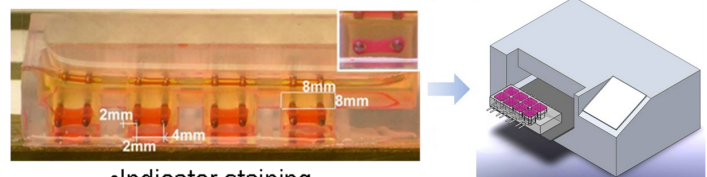
MMP is normally maintained at a steady level in healthy cells. Disruptions of MMP can lead to cellular damage and eventually tissue and organ dysfunction. Dissipation of MMP is an early indicator of cellular apoptosis. Precise quantification of MMP can thus be used to assess potential toxic effects of test compounds.

### Technical Advantages:

- ◆ Rapidly assess mitochondrial membrane potential (MMP)
- ◆ Dramatically improve the detection sensitivity of TMRE (and thus MMP) signals in cell- and tissue-based assays
- ◆ Quantify the physiology of cells grown in a more natural three-dimensional (3D) environment
- ◆ Correlate MMP levels with multiple parameters of cell physiology including cellular viability and contractility assessed by MTT and InvivoSciences, LLC's proprietary Palpator™ technology
- ◆ Culture and repeatedly assess 3D tissue constructs for days and weeks in prolonged experiments and achieve accurate results

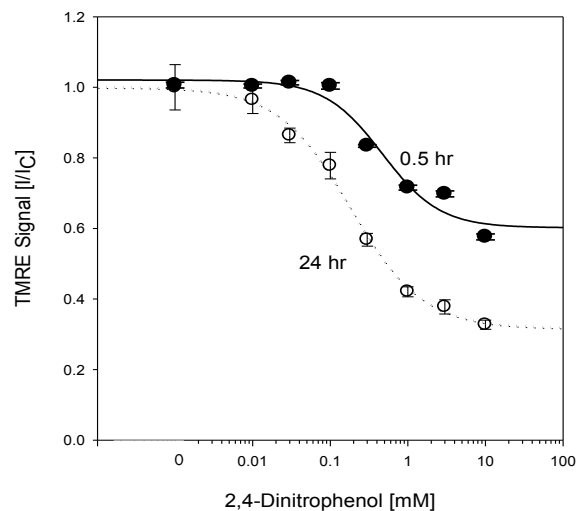
### Example:

Fibroblasts embedded in 3D tissue constructs were labeled with 500 nM TMRE for 30 minutes and then treated with 2,4-dinitrophenol (DNP), a MMP un-coupler. TMRE signal was quantified using a plate reader (see below).



- Indicator staining
- Drug treatment

Signal reading



DNP treatment dose-dependently reduced MMP within 30 minutes and continued to reduce it over a 24 hour period (figure above). The EC<sub>50</sub> of DNP was estimated to be 0.47  $\mu$ M at 30 minutes and 0.18  $\mu$ M at 24 hours. These results demonstrate that DNP's acute and long-term effect on MMP can be precisely quantified using 3D tissue constructs.

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