

# MitoXpress Xtra Oxygen Consumption Assay

## Instrument setup guide for Tecan plate readers

### Recommendations for Infinite F Nano<sup>+</sup>/F Plex/F200 PRO plate readers

The Tecan Infinite F Nano<sup>+</sup>/F Plex/F200 PRO allows the use of the MitoXpress Xtra Assay with dual-read time resolved fluorescence (TRF) lifetime detection using well-wise kinetic measurements of two labels with different TRF lag times. These two TRF intensities allow the ratio-metric calculation of fluorescence lifetimes representing the level of oxygen in each sample.

Infinite F Nano<sup>+</sup>/F Plex/F200 PRO readers must be equipped with suitable filter sets. For optimal performance, we recommend using dual-read TRF lifetime detection, and setting up the measurement conditions in the instrument control software according to the following instructions.

To create a new Protocol in Tecan Control software:

1. Insert a 'Kinetic' Loop strip.
2. Insert a 'Well wise' measurement strip indented once to the right.
3. Insert two Detection 'Label' strips below the Well strip, both indented once to the right.
4. Set the parameters of each action, as described in the following instrument setup table:

#### Example 'list of actions' entered in a new Protocol

List of actions		
Kinetic		
Fluorescence		
	Measurement well-wise	
		Label 1
		Label 2

Please refer to the Tecan instrument software user manual for further information.

### Instrument setup

Method	Dual-Read TRF
No. of labels	Two
Kinetic duration	02:00:00 (two hours)
Interval time	Minimal
Kinetic measurement	Well-wise
Measurement mode	TRF Intensity bottom (preferred) or top
Excitation filter	380 (20) nm
Emission filter	670 (40) nm
Settle time	0 ms
No. of flashes	25
Gain	160*
Lag time label 1	30 µs
Integration time label 1	30 µs
Lag time label 2	70 µs
Integration time label 2	30 µs

\* Optimal gains can differ, and should be optimized for each specific plate reader model. For correct lifetime calculations, those parameters must be identical for both lag times.

## Recommendations for SPARK plate readers

The Tecan Spark allows the use of the MitoXpress Xtra Assay with dual-read TRF lifetime detection using well-wise kinetic measurements of two labels with different TRF lag times. These two TRF intensities allow the ratio-metric calculation of fluorescence lifetimes, representing the level of oxygen in each sample.

Spark readers must be equipped with suitable filter sets. For optimal performance, we recommend using dual-read TRF lifetime detection, and setting up the measurement conditions in the instrument control software according to the following instructions.

To create a new Protocol in Tecan Control software:

1. Insert a 'Plate' strip and 'Temperature' strip.
2. Insert a 'Kinetic' Loop strip.
3. Insert a 'Well' measurement strip indented once to the right.
4. Insert two Detection 'Label' strips below the Well strip, both indented once to the right.
5. Set the parameters of each action, as described in the following instrument setup table:

### Example 'list of actions' entered in a new Protocol

List of actions			
Plate			
	Temperature		
	Kinetic		
	Well		
		TRF Intensity	30 µsec delay
		TRF Intensity	70 µsec delay

Please refer to the Tecan instrument software user manual for further information.

### Instrument setup

Method	Dual-Read TRF
No. of labels	Two
Kinetic duration	02:00:00 (two hours)
Interval time	Minimal
Kinetic measurement	Well-wise
Measurement mode	TRF Intensity bottom (preferred) or top
Excitation	380 (20) nm Filter (or Monochromator Fusion Optics)
Emission filter	670 (40) nm (Filter only)
Settle time	0 ms
Z-Position mode	Manual
Z-Position	28,000 µm*
No. of flashes	30
Gain	90 (Filter) or 110 (Monochromator)*
Lag time label 1	30 µs
Integration time label 1	30 µs
Lag time label 2	70 µs
Integration time label 2	30 µs

\* Optimal gains and Z-positions can differ, and should be optimized for each specific plate reader model. For correct lifetime calculations, those parameters must be identical for both lag times.

## Recommendations for Infinite M Plex/ M Nano<sup>+</sup>/M200PRO plate readers

The Tecan Infinite M Nano<sup>+</sup>/M Plex/M200PRO allows the use of the MitoXpress Xtra Assay with single-read TRF detection. The TRF intensities allow the ratio-metric calculation of fluorescence lifetimes, representing the level of oxygen in each sample.

For optimal performance, we recommend setting up the measurement conditions for Infinite M Nano<sup>+</sup>/M Plex/M200PRO readers in the instrument control software according to the following instructions.

To create a new Protocol in Tecan Control software:

1. Insert a 'Kinetic' Loop strip.
2. Insert a 'Well wise' measurement strip indented once to the right.
3. Insert one Detection 'Label' strip below the Well strip, indented once to the right.
4. Set the parameters of each action, as described in the following instrument setup table:

### Example 'list of actions' entered in a new Protocol

List of actions		
Kinetic		
Fluorescence		
	Measurement well-wise	
		Label 1

Please refer to the Tecan instrument software user manual for further information.

### Instrument setup

Method	TRF
No. of labels	One
Kinetic duration	02:00:00 (two hours)
Interval time	Minimal
Kinetic measurement	Well-wise
Measurement mode	TRF Intensity bottom (preferred) or top
Excitation	380 (9) nm
Emission	650 (20) nm
Settle time	0 ms
No. of flashes	25
Gain	Manual 180*
Lag time label 1	30 µs
Integration Time label 1	100 µs

\* Optimal gains can differ, and should be optimized for each specific plate reader model.

## Recommendations for Infinite M1000 PRO plate readers

The Tecan Infinite M1000PRO allows the use of the MitoXpress Xtra Assay with single-read TRF detection. The TRF intensities allow the ratio-metric calculation of fluorescence lifetimes, representing the level of oxygen in each sample.

For optimal performance, we recommend setting up the measurement conditions for Infinite M1000PRO readers in the instrument control software according to the following instructions.

To create a new Protocol in Tecan Control software:

1. Insert a 'Kinetic' Loop strip.
2. Insert a 'Well wise' measurement strip indented once to the right.
3. Insert one Detection 'Label' strip below the Well strip, indented once to the right.
4. Set the parameters of each action, as described in the following instrument setup table:

### Example 'list of actions' entered in a new Protocol

List of actions		
Kinetic		
Fluorescence		
	Measurement well-wise	
		Label 1

Please refer to the Tecan instrument software user manual for further information.

### Instrument setup

Method	TRF
No. of Labels	One
Kinetic duration	02:00:00 (two hours)
Interval time	Minimal
Kinetic measurement	Well-wise
Measurement mode	TRF Intensity bottom (preferred) or top
Excitation	380 (9) nm
Emission	650 (20) nm
Settle time	0 ms
No. of flashes	20
Flash frequency	100 Hz
Gain	Manual 130*
Z-Position	28,000 $\mu\text{m}^*$
Lag time label 1	30 $\mu\text{s}$
Integration time label 1	100 $\mu\text{s}$

\* Optimal gains and Z-positions can differ, and should be optimized for each specific plate reader model.

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