Data Sheet

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pH-Xtra Glycolysis Assay

Instrument setup guide for Tecan plate readers

Recommendations for Infinite F Nano⁺/ F Plex/F200 PRO plate readers

The Tecan Infinite F Nano⁺/F Plex/F200 PRO permits the use of the pH-Xtra Glycolysis Assay with dual-read time-resolved fluorescence (TRF) lifetime detection using two TRF measurements with different TRF lag times. These two TRF intensities allow the ratio-metric calculation of fluorescence lifetimes representing the level of extracellular acidification in each sample.

Infinite F Nano⁺/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	TR Fluorescence Intensity Top
Excitation filter	380 (20) nm
Emission filter	620 (20) nm
Mirror	Dichroic 510
Settle time	0 ms
No. of flashes	25
Gain	150*
Lag time Label 1	100 µs
Integration time Label 1	30 µs
Lag time Label 2	300 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, parameters must be identical for both lag times.

Recommendations for SPARK plate readers

The Tecan Spark allows the use of the pH-Xtra Glycolysis Assay with dual-read TRF lifetime detection using two TRF measurements with different TRF lag times. These two TRF intensities allow the ratio-metric calculation of fluorescence lifetimes representing the level of extracellular acidification 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	100 µsec delay
		TRF Intensity	300 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	TR Fluorescence Intensity Top
Excitation	380 (20) nm Filter (or Monochromator Fusion Optics)
Emission filter	620 (20) nm (Filter only)
Settle time	0 ms
Z-Position mode	Manual
Z-Position	32,000 µm*
No. of Flashes	30
Gain	110*
Lag time Label 1	100 µs
Integration time Label 1	50 µs
Lag time Label 2	300 µs
Integration time Label 2	50 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+/M200PRO plate readers

The Tecan Infinite M Nano+/M Plex/M200PRO allows the use of the pH-Xtra Glycolysis Assay with TRF detection. The TRF intensities measured are proportional to the level of extracellular acidification in each sample.

For optimal performance, we recommend setting up the measurement conditions for Infinite M Nano+/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 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, Top read
Excitation	380 (9) nm
Emission	615 (20) nm
Settle time	0 ms
Z-Position mode	Manual
Z-Position	20,500 µm*
No. of flashes	25
Gain	Manual 200*
Lag time	100 µs
Integration time	100 µs

* Optimal gains and Z-positions 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 pH-Xtra Glycolysis Assay with TRF detection. The TRF intensities measured are proportional to the level of extracellular acidification 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 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, Top read
Excitation	380 (20) nm
Emission	615 (20) nm
Settle time	0 ms
No. of flashes	20
Z-Position mode	Manual
Z-Position	20,500 µm*
Flash frequency	100 Hz
Gain	Manual 130*
Lag time	100 µs
Integration time	100 µs

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

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