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pH-Xtra Glycolysis Assay

Instrument setup guide for Perkin Elmer plate readers

Recommendations for Victor 3/X4/X5 plate readers

The Perkin Elmer Victor 3/X4/X5 plate reader with TRF capability allows the use of the pH-Xtra Glycolysis 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 extracellular acidification in each sample.

Perkin Elmer Victor 3/X4/X5 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.

1. Create a Protocol in the Perkin Elmer 2030 Explorer Application.

2. Open the Protocol and add a TRF Label in the Measurement Tab.

3. Edit the Properties of the TRF Label to set the parameters as described in the following Instrument setup table.

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

Instrument setup

Method	Dual-Read TRF
Measure each plate	120 times (assuming 96-well measurement)
Delay between repeats	0
Plate type	Generic 8 × 12 size plate
Measurement mode	TRF Label
Excitation filter	D340
Emission filter	D615
Flash energy mode	High
Flash energy level	100
Light exposure time	Long
Light exposure ref. level	30
Counting delay 1	100 µs
Counting window 1	30 µs
Counting delay 2	300 µs
Counting window 2	30 µs
Counting cycle	1,000
Beam size	Normal
Second measurement	N/A

* Optimal flash energy level and light exposure ref level can differ, and should be optimized for each specific plate reader model. The counting delay and window times should not be adjusted from these defined values.

Recommendations for EnVision plate readers

The Perkin Elmer EnVision plate reader with TRF capability allows to use the pH-Xtra Glycolysis 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 extracellular acidification in each sample.

Perkin Elmer EnVision 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.

1. Create a TRF Label in the Wallac Envision Manager

2. Edit the Properties of the Label to set the parameters as described in the following Instrument setup table.

3. Create a Protocol in the Envision Manager and select to use the new Label.

4. **Caution—important for data analysis:** Use only the RFU signal outputted for Sequential Windows No. 1 and No. 5 for the dual-read ratio-metric lifetime calculations

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

Instrument setup

Method	Dual-Read TRF
Number of plate repeats	120 times (assuming 96-well measurement)
Start plate repeat each	2 minutes
Plate type	96 General
Measurement mode	TRF Label
Excitation	Top
Emission	Top
Light source	Flash Lamp
Top mirror	LANCE/DELFIA
Bottom mirror	None
Excitation filter	UV (TRF) 340
Emission filter	615 nm
2nd Emission filter	N/A
Use of 2nd emission filter	Not used
Measurement height	6.5 mm
Excitation light	100 %
Delay	100
Window time	50
Number of sequence windows	5
Time between flashes	2,000
Number of flashes	30

* Optimal flash number can differ, and should be optimized for each specific plate reader model. The delay and window times should not be adjusted from these defined values.

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