Data Sheet

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pH-Xtra Glycolysis Assay

Instrument Set Up Guide for BioTek Plate Readers

Recommendations for Cytation 1/3/5 plate readers

The BioTek Cytation 1/3/5 allows use of the pH-Xtra Glycolysis Assay with dual-read time-resolved fluorescence (TRF) lifetime detection using a kinetic loop with two TRF measurements with different TRF delay times. These two TRF intensities allow the ratio-metric calculation of fluorescence lifetimes, which represent the level of extracellular acidification in each sample.

Cytation 1/3/5 readers must be equipped with suitable filter cubes. 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 parameters. Create a Gen5 Protocol with the following Step actions: a Kinetic Step containing two distinct TRF Read actions, Read 1 and Read 2 settings described below. Alternatively, preconfigured Gen5 assay protocols with the default settings are available for download from the BioTek website.

Instrument setup

Method	Dual-read TRF
Mode	Kinetic step (2x TR-Fluorescence Endpoint)
Interval time	Minimal
Light source	Xenon flash lamp
Lamp energy	High
Excitation filter	360/40 nm
Emission filter	620/10 nm
Mirror	400 nm
Optics position	Top
Read height	Default for Top read: 5.25 mm, volume and plate format dependent*
Read speed	Normal (Delay 100 msec)
Measurements per data point	10
Gain	110*
Delay read 1	100 µs
Collection time 1	30 µs
Delay read 2	300 µs
Collection time 2	30 µs

* Optimal gains and read height can differ, and should be optimized for each experiment. For correct lifetime calculations, those parameters must be identical for both delay times.

BioTek Filter Cube part numbers

- 1035124 (CUBE 124 PH-XTRA GLYCOLYSIS ASBY)
- 8040588 (FLTR BLK PH-XTRA GLYCOLYSIS ASBY)

Recommendations for Synergy H1/H4 plate readers

The BioTek Synergy H1/H4 allows use of the pH-Xtra Glycolysis Assay with dual-read TRF lifetime detection using a kinetic loop with two TRF measurements with different TRF delay times. These two TRF intensities allow the ratio-metric calculation of fluorescence lifetimes, which represent the level of extracellular acidification in each sample.

Synergy H1/H4 readers must be equipped with suitable filter cubes. 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 parameters. Create a Gen5 Protocol with the following Step actions: a Kinetic Step containing two distinct TRF Read actions, Read 1 and Read 2 settings described below. Alternatively, preconfigured Gen5 assay protocols with the default settings are available for download from the BioTek website.

Instrument setup

Method	Dual-read TRF
Mode	Kinetic step (2x TR-Fluorescence Endpoint)
Interval time	Minimal
Light source	Xenon flash lamp
Lamp energy	High
Excitation filter	360/40 nm
Emission filter	620/10 nm
Mirror	400 nm
Optics position	Top
Read height	Default for Top read: 5.25 mm, volume and plate format dependent*
Read speed	Normal (Delay 100 msec)
Measurements per data point	10
Gain	110*
Delay read 1	100 µs
Collection time 1	30 µs
Delay read 2	300 µs
Collection time 2	30 µs

* Optimal gains and read height can differ, and should be optimized for each experiment. For correct lifetime calculations, those parameters must be identical for both delay times.

BioTek Filter Cube part numbers

- 1035124 (CUBE 124 PH-XTRA GLYCOLYSIS ASBY)
- 8040588 (FLTR BLK PH-XTRA GLYCOLYSIS ASBY)

Recommendations for Synergy Neo/Neo2 plate readers

The BioTek Synergy Neo/Neo2 allows use of the pH-Xtra Glycolysis Assay with dual-read TRF lifetime detection using a kinetic loop with two TRF measurements with different TRF delay times. These two TRF intensities allow the ratio-metric calculation of fluorescence lifetimes, which represent the level of extracellular acidification in each sample.

Synergy Neo/Neo2 readers must be equipped with suitable filter cubes. 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 parameters. Create a Gen5 Protocol with the following Step actions: a Kinetic Step containing two distinct TRF Read actions, Read 1 and Read 2 settings described below. Alternatively, preconfigured Gen5 assay protocols with the default settings are available for download from the BioTek website.

Instrument setup

Method	Dual-read TRF
Mode	Kinetic step (2x TR-Fluorescence Endpoint)
Interval time	Minimal
Light source	Xenon flash lamp
Lamp energy	High
Excitation filter	360/40 nm
Emission filter	620/10 nm
Mirror	400 nm
Optics position	Top or bottom
Read height	Default for Top read: 5.25 mm 10 mw (S/B), volume and plate format dependent*
Read speed	Normal (Delay 100 msec)
Measurements per data point	10
Gain	100*
Delay read 1	100 µs
Collection time 1	30 µs
Delay read 2	300 µs
Collection time 2	30 µs

* Optimal gains and read height can differ, and should be optimized for each experiment. For correct lifetime calculations, those parameters must be identical for both delay times.

BioTek Filter Cube part numbers

- 1035124 (CUBE 124 PH-XTRA GLYCOLYSIS ASBY)
- 8040588 (FLTR BLK PH-XTRA GLYCOLYSIS ASBY)

Gen5 v3.04 Data Analysis Protocol File name

AgilentpHX.prt

BioTek Filter Cube part numbers

- 1035124 (CUBE 124 PH-XTRA GLYCOLYSIS ASBY)
- 8040588 (FLTR BLK PH-XTRA GLYCOLYSIS ASBY)

Application Bulletin

BioTek Toolkit for Agilent pH Xtra assay

BioTek User Tutorial

Agilent pH-XTRA Glycolysis Assay User Tutorial

www.agilent.com/chem/discoverXF

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