Data Sheet

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pH-Xtra Glycolysis Assay

Instrument Set Up Guide for BMG Plate Readers

Recommendations for CLARIOstar plate readers

CLARIOstar readers must be equipped with suitable filter sets.

Filter part numbers

- **Excitation filter:** Ex TR
- **Emission filter:** BP615-18
- **Dichroic mirror:** LP-TR

The CLARIOstar software supplied by BMG LABTECH should include default assay protocols and analysis templates for pH-Xtra products, but if not visible, this guide will allow users to set up a new protocol.

For optimal performance, we recommend using dual-read time-resolved fluorescence (TRF) lifetime detection and setting up the measurement conditions in the instrument control software according to the following parameters. The two TRF intensities collected allow the ratio-metric calculation of fluorescence lifetimes, which represent the extracellular acidification in each sample.

Instrument Control Protocols and MARS data analysis templates are available.

For more information, visit:
www.bmglabtech.com/contact

Instrument setup—TRF mode (recommended)

Method	TRF
Mode	Plate mode
Microplate	BMG LABTECH 96
Focal height	Default for Bottom read: 5.5 mm volume and plate format dependent*
Optic settings	
Optic	Bottom or Top
No. of multichromatics	2
Well multichromatics	On
Gain	2,100*
Multichromatic settings	
Excitation filter	ExTR (340 ±50 nm), set in optic no. 1 and 2
Emission filter	BP615-18, set in optic no. 1 and 2
Dichroic filter	LP-TR, set in optic no. 1 and 2
Multichromatics	
Integration start 1 Integration start 2	100 µs 300 µs
Integration time 1 Integration time 2	30 µs 30 µs
General	
Setting time	0.1
No. of kinetic windows	1
Kinetic window 1	
No. of cycles	90, if using 60 seconds cycle time
No. of flashes per well per cycle	100
Cycle time	60 seconds, dependent on layout

* A default gain of 2100 is given, but optimal gains and focal height can differ for each instrument; therefore, if saturation is observed, optimizing the gain may be required. For correct lifetime calculations, those parameters must be identical for both multichromatics.

Recommendations for FLUOstar/POLARstar Omega plate readers

Omega instruments equipped with fluorescence intensity (FI) must be equipped with suitable filter sets.

Filter part numbers

- **Excitation filter:** Ex TR L
- **Emission filter:** BP615-18

The Omega software supplied by BMG LABTECH should include default assay protocols and analysis templates for pH-Xtra products, but if not visible, this guide will allow users to set up a new protocol.

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. The two TRF intensities collected allow the ratio-metric calculation of fluorescence lifetimes, which represent the extracellular acidification in each sample.

Instrument —TRF mode (recommended)

Method	TRF
Mode	Plate mode
Microplate	BMG LABTECH 96
Focal height	TR-F Optical attachment set: 6 mm volume and plate format dependent*
Optic settings	
Optic	Advanced Optic Head for TRF
No. of multichromatics	2
Well multichromatics	On
Gain	2,300*
Multichromatic settings	
Excitation filter	TR EX-L (340 ±50 nm), set in optic no. 1 and 2
Emission filter	BP655 (615 ±18 nm) , set in optic no. 1 and 2
Multichromatics	
Integration start 1 Integration start 2	100 µs 300 µs
Integration time 1 Integration time 2	30 µs 30 µs
General	
Setting time	0.3
No. of kinetic windows	1
Kinetic window 1	
No. of cycles	90, if using 60 seconds cycle time
Measurement start time	0.0
No. of flashes per well per cycle	50
Cycle time	60 seconds, dependent on layout

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

Instrument Control Protocols and MARS data analysis templates are available.

For more information, visit:
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Recommendations for PHERAstar FS/FSX plate readers

PHERAstar FS/FSX readers equipped with TRF detection must be equipped with the appropriate optic module. Either the HTRF or TRF 337 615 optic module may be used. Measurement conditions should be configured in the instrument control software according to the following parameters.

The two TRF intensities collected allow the ratio-metric calculation of fluorescence lifetimes, which represent the extracellular acidification in each sample.

Instrument setup—TRF mode (recommended)

Method	TRF
Mode	Plate mode
Microplate	BMG LABTECH 96
Focal height	Default for Bottom read: 7 mm volume and plate format dependent*
Optic settings	
Optic	Bottom or Top
No. of multichromatics	2
Well multichromatics	On
Simultaneous dual emission	Off
Optic Module	HTRF module or TRF 337 615 module
Integration start	100
Integration time	30
Excitation source	Flash lamp
Multichromatic settings	
Excitation filter	HTRF or TRF 337 615 module (337 nm), set in optic no. 1 and 2
Emission filter	HTRF (620 nm)or TRF 337 615 (615 nm), set in optic no. 1 and 2
Multichromatics	
Integration start 1 Integration start 2	100 µs 300 µs
Integration time 1 Integration time 2	30 µs 30 µs
General	
Setting time	0.3
No. of kinetic windows	1
Kinetic window 1	
No. of cycles	90, if using 60 seconds cycle time
No. of flashes per well per cycle	100
Cycle time	60 seconds, dependent on layout

* Optimal focal height can differ, and should be optimized for each experiment.

Instrument Control Protocols and MARS data analysis templates are available.

For more information, visit:
www.bmglabtech.com/contact

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