Data Sheet

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MitoXpress Xtra Oxygen Consumption 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:** BP645-20
- **Dichroic mirror:** LP-TR

The CLARIOstar software supplied by BMG LABTECH should include default assay protocols and analysis templates for MitoXpress products but if not visible then 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 level of oxygen in each sample.

Instrument Control Protocols and MARS data analysis templates are available.

For more information, visit:

www.bmglabtech.com/contact

Instrument setup—TRF (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 (preferred) or Top
No. of multichromatics	2
Well multichromatics	On
Gain	2,300*
Multichromatic settings	
Excitation filter	ExTR (340 ±50 nm), set in optic no. 1 and 2
Emission filter	BP645-20, 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	30 µs 70 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 2,300 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, the gain parameter 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:** BP645-20

The Omega software supplied by BMG LABTECH should include default assay protocols and analysis templates for MitoXpress 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 level of oxygen in each sample.

Instrument setup—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	2,400*
Multichromatic settings	
Excitation filter	TR EX-L (340 ±50 nm), set in optic no. 1 and 2
Emission filter	BP655 (655 ±50 nm), set in optic no. 1 and 2
Multichromatics	
Integration start 1 Integration start 2	30 µs 70 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.

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Recommendations for PHERAstar FS/FSX plate readers

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

Instrument setup—TRF mode (recommended)

Method	TRF
Mode	Plate mode
Microplate	BMG LABTECH 96
Focal height	TRF Optical attachment set: 4.5 mm volume and plate format dependent*
Optic settings	
Optic	Bottom optic
No. of multichromatics	1
Simultaneous dual emission	Off
Optic module	HTRF module
Excitation filter	HTRF module (337 nm)
Emission filter	HTRF module (655 nm)
Integration start	40 µs
Integration time	100 µs
Excitation source	Flash lamp
General	
Setting time	0.3
No. of kinetic windows	1
No. of cycles	90, if using 60 seconds cycle time
Measurement start time	0.0
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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