Data Sheet

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MitoXpress Xtra Oxygen Consumption Assay

Instrument Set Up Guide for Molecular Devices Plate Readers

Recommendations for SpectraMax i3/i3x plate readers

The Molecular Devices SpectraMax i3/i3x allows use of the MitoXpress Xtra Assay with time-resolved fluorescence (TRF) detection. The TRF intensities measured are proportional to the level of oxygen in each sample.

SpectraMax i3/i3x readers must be equipped with the suitable filter cartridge. For optimal performance, we recommend setting up the measurement conditions in the instrument control software according to the following parameters. Alternatively, preconfigured SoftMax Pro assay protocols with the default settings are available for download from the SoftMaxPro.org website.

Instrument setup—TRF mode (recommended)

Read mode	TRF
Light source	LED
Read type	Kinetic, Top read
Detection module	TRF-EuSa Filter Cartridge
Excitation filter	370 nm
Emission filter	642-BW10 nm
No. of pulses	100*
Excitation time	50 µs
Integration time	100 µs
Delay time	30 µs
Read height	Optimized for each plate type using Z-height optimization tool

* Optimal pulse numbers can differ, and should be optimized for each experiment.

If no suitable filter cartridge is available, the MitoXpress Assay can alternatively be measured using nonTRF intensity (FI) measurements on SpectraMax i3/i3x readers using the settings below. For optimal assay performance, we strongly recommend using TRF mode.

Alternative instrument setup—FI mode

Read mode	FI
Light source	Xenon flash lamp
Read type	Kinetic, Top read
Detection module	Monochromator
Excitation filter	380-BW15 nm
Emission filter	650-BW15 nm
No. of flashes	10*
PMT setting	High
Read height	Optimized for each plate type using Z-height optimization tool

* Optimal flash numbers can differ, and should be optimized for each experiment.

Cartridge part number

TRF EuSa Detection Cartridge: 0200-7008

SoftMax Pro Protocol and data analysis templates are available.

For more information, visit:

www.softmaxpro.org

Recommendations for SpectraMax Paradigm plate readers

The Molecular Devices SpectraMax Paradigm allows use of the MitoXpress Xtra Assay with single-read TRF detection. The TRF intensities measured are proportional to the level of oxygen in each sample.

SpectraMax Paradigm readers must be equipped with a suitable filter cartridge. For optimal performance, we recommend setting up the measurement conditions in the instrument control software according to the following parameters. Alternatively, preconfigured SoftMax Pro assay protocols with the default settings are available for download from the SoftMaxPro.org website.

Instrument setup—TRF mode (recommended)

Read mode	TRF
Light source	LED (TRF-EuSa Filter Cartridge)
Read type	Kinetic, Bottom read (preferred) or Top read
Detection module	TRF-EuSa Filter Cartridge
Excitation filter	370 nm
Emission filter	642-BW10 nm
No. of pulses	100*
Excitation time	50 µs
Integration time	100 µs
Delay time	30 µs
Read height	Optimized for each plate type using Z-height optimization tool

* Optimal pulse numbers can differ, and should be optimized for each experiment.

If no suitable filter cartridge is available, the MitoXpress Assay can alternatively be measured using FI measurements on SpectraMax Paradigm readers using the TUNE cartridge and the instrument settings below.

For optimal assay performance, we strongly recommend using TRF mode.

Alternative instrument setup—FI mode

Read mode	FI
Light source	LED
Read type	Kinetic, Bottom read (preferred) or Top read
Detection module	TUNE detection cartridge
Excitation filter	380-BW15 nm
Emission filter	650-BW15 nm
Integration time	400 ms
PMT	High
On-the-fly detection	Off
Read height	Optimized for each plate type using Z-height optimization tool

* Optimal flash numbers can differ, and should be optimized for each experiment.

Cartridge part numbers

- TRF EuSa Detection Cartridge: 0200-7008
- TUNE Detection Cartridge: 0200-7050

SoftMax Pro Protocol and data analysis templates are available.

For more information, visit:
www.softmaxpro.org

Recommendations for SpectraMax iD5 plate readers

The Molecular Devices SpectraMax iD5 allows use of the MitoXpress Xtra Assay with single-read TRF detection. The TRF intensities measured are proportional to the level of oxygen in each sample.

SpectraMax iD5 readers must be appropriately configured by setting up the measurement conditions in the instrument control software according to the following parameters. The MitoXpress Xtra Assay can be measured using filter- or monochromator-based optics on the SpectraMax iD5.

We recommend using filter-based optics for optimal performance where available.

Instrument setup—TRF mode

Read mode	TRF
Light source	Xenon flash lamp
Read type	Kinetic, Bottom (preferred) or Top Read
Detection module	Filter (preferred) or Monochromator
Excitation	350-BW60 nm (Filter) 380-BW15 nm (Monochromator)
Emission	642-BW10 nm (Filter) 650-BW25 nm (Monochromator)
No. of pulses	100*
Excitation time	50 µs
Integration time	100 µs
Delay time	30 µs
Read height	Optimized for each plate type using Z-height optimization tool, fixed to bottom of plate for bottom read

* Optimal pulse numbers can differ, and should be optimized for each experiment.

Filter part numbers

- 350nm BW 60nm: 6590-0081—comes as standard with iD5
- 642nm BW 10nm: 6590-0120

SoftMax Pro Protocol and data analysis templates are available.

For more information, visit:

www.softmaxpro.org

Recommendations for SpectraMax M series 2-5e and FlexStation 3 plate readers

The Molecular Devices SpectraMax M series 2-5e and FlexStation 3 allow use of the MitoXpress Xtra Assay with FI detection. The FIs measured are proportional to the level of oxygen in each sample.

SpectraMax M series 2-5e and FlexStation 3 readers must be appropriately configured by setting up the measurement conditions in the instrument control software according to the following parameters.

Instrument setup—FI mode

Read mode	FI
Light source	Xenon flash lamp
Read type	Kinetic, Bottom (preferred where available) or Top Read
Detection module	Monochromator
Excitation	380-BW9 nm
Cut off	630 nm
Emission	650-BW15 nm
No. of flashes	30*
PMT	High

* Optimal flash numbers can differ, and should be optimized for each experiment.

Recommendations for SpectraMax Gemini XPS/EM plate readers

The Molecular Devices SpectraMax Gemini XPS/EM allows use of the MitoXpress Xtra Assay with FI detection. The FIs measured are proportional to the level of oxygen in each sample.

SpectraMax Gemini XPS/EM readers must be appropriately configured by setting up the measurement conditions in the instrument control software according to the following parameters.

Instrument setup—FI mode

Read mode	FI
Light source	Xenon flash lamp
Read type	Kinetic, Bottom (Gemini EM) or Top Read (Gemini XPS)
Detection module	Monochromator
Excitation	380-BW9 nm
Cut off	630 nm
Emission	650-BW9 nm
No. of flashes	30*
PMT	High

* Optimal pulse numbers can differ, and should be optimized for each experiment.

Recommendations for SpectraMax Gemini iD3 plate readers

The Molecular Devices SpectraMax iD3 allows use of the MitoXpress Xtra Assay with FI detection. The FIs measured are proportional to the level of oxygen in each sample.

SpectraMax iD3 readers must be appropriately configured by setting up the measurement conditions in the instrument control software according to the following parameters.

Instrument setup—FI mode

Read mode	FI
Light source	Xenon flash lamp
Read type	Kinetic, Bottom (preferred) or Top Read
Detection module	Monochromator
Excitation	380-BW15 nm
Emission	650-BW25 nm
Integration time	400 ms
PMT	High
Read height	Optimized for each plate type using Z-height optimization tool, fixed to bottom of plate for bottom read

* Optimal flash numbers can differ, and should be optimized for each experiment.

SoftMax Pro Protocol and data analysis templates are available.

For more information, visit:

www.softmaxpro.org

www.agilent.com/chem/discoverXF

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