Data Sheet

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pH-Xtra Glycolysis 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 pH-Xtra Glycolysis Assay with time-resolved fluorescence (TRF) detection. The TRF intensities measured are proportional to the level of extracellular acidification in each sample.

SpectraMax i3/i3x 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) Xenon flash lamp (HTRF Cartridge)
Read type	Kinetic, Top read
Detection module	TRF-EuSa Filter Cartridge (recommended) or HTRF Cartridge
Excitation filter	370 nm (TRF-EuSa Filter Cartridge) 340 nm (HTRF Cartridge)
Emission filter	616-BW10 nm (TRF-EuSa Filter Cartridge) 616-BW10 nm (HTRF Cartridge)
No. of pulses	100* (TRF-EuSa Filter Cartridge) 50* (HTRF Cartridge)
Excitation time	50 µs
Integration time	100 µs
Delay time	100 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.

Cartridge part numbers

- TRF EuSa Detection Cartridge: 0200-7008
- HTRF Detection Cartridge: 0200-7011

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 pH-Xtra Glycolysis Assay with TRF detection. The TRF intensities measured are proportional to the level of extracellular acidification 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) Xenon flash lamp (HTRF Cartridge)
Read type	Kinetic, Bottom read (preferred) or Top read
Detection module	TRF-EuSa Filter Cartridge (recommended) or HTRF Cartridge
Excitation filter	370 nm (TRF-EuSa Filter Cartridge) 340 nm (HTRF Cartridge)
Emission filter	616-BW10 nm (TRF-EuSa Filter Cartridge) 616-BW10 nm (HTRF Cartridge)
No. of pulses	100* (TRF-EuSa Filter Cartridge) 50* (HTRF Cartridge)
Excitation time	50 µs
Integration time	100 µs
Delay time	100 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.

Cartridge part numbers

- TRF EuSa Detection Cartridge: 0200-7008
- HTRF Detection Cartridge: 0200-7011

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 pH-Xtra Glycolysis Assay with TRF detection. The TRF intensities measured are proportional to the level of extracellular acidification 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 pH-Xtra Glycolysis 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 (recommended)

Read mode	Time resolved fluorescence (TRF)
Light source	Xenon flash lamp
Read type	Kinetic, Bottom read (preferred) or Top read
Detection module	Filter (recommended) or Monochromator
Excitation	350-BW60 nm (Filter) 380-BW15 nm (Monochromator)
Emission	616-BW10 nm (Filter) 615-BW25 nm (Monochromator)
No. of pulses	100*
Excitation time	50 µs
Integration time	100 µs
Delay time	100 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: 6590-008 (comes standard with iD5)
- 616nm BW 10nm: 6590-0118 (comes standard with iD5)

Recommendations for SpectraMax M series 4-5e and FlexStation 3 plate readers

The Molecular Devices SpectraMax M series 4-5e and FlexStation 3 allow use of the pH-Xtra Glycolysis Assay with TRF detection. The TRF intensities measured are proportional to the level of extracellular acidification in each sample.

SpectraMax M series 4-5e 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—TRF mode (recommended)

Read mode	TRF
Light source	Xenon flash lamp
Read type	Kinetic, Bottom (preferred where available) or Top Read
Detection module	Monochromator
Excitation	380-BW9 nm
Cut off	590 nm
Emission	615-BW15 nm
No. of pulses	100*
Integration time	100 µs
Delay time	100 µs

* Optimal pulse 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 allow use of the pH-Xtra Glycolysis Assay with TRF detection. The TRF intensities measured are proportional to the level of extracellular acidification 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—TRF mode (recommended)

Read mode	TRF
Light source	Xenon flash lamp
Read type	Kinetic, Bottom (preferred where available) or Top Read
Detection module	Monochromator
Excitation	380-BW9 nm
Cut off	590 nm
Emission	615-BW15 nm
No. of pulses	100*
Integration time	100 µs
Delay time	100 µs

* 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

SoftMax Pro Protocol and data analysis templates are available.

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www.softmaxpro.org

www.agilent.com/chem/discoverXF

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