

**HTRF<sup>®</sup> Europium cryptate donor / Red acceptor readout  
Setup recommendations for SpectraMax<sup>®</sup> M5<sup>e</sup>**

Two sequential measurements should be carried out: at 620 nm for the cryptate emission, and at 665 nm for the specific signal emitted by the acceptor (XL665 or d2). The ratio of the two fluorescence intensities 665/620 (acceptor/donor) enables the calculation of Delta F (%) which represents the relative energy transfer rate for each sample.

SpectraMax<sup>®</sup> M5e readers must be appropriately configured for HTRF<sup>®</sup> readout by setting up the measurement conditions in the SoftMax Pro software. In particular, these parameters should be entered as defined in the table below. No special upgrade is required for HTRF<sup>®</sup> readout, as it is a monochromator-based instrument.

<b>Read mode</b>	
	Time-Resolved Fluorescence (RFUs)
Integration delay	50 µs
Integration	400 µs
	Top read
<b>Wavelengths</b>	
Excitation 1	314 nm
Emission 1	665 nm
Cut off for emission 1	630 nm
Excitation 2	314 nm
Emission 2	620 nm
Cut off for emission 2	570 nm
<b>Sensitivity</b>	
Readings	50 to 100
PMT	Optimal
<b>Column Wavelength</b>	
	Column Priority

**This reader only allows high performance HTRF measurement when assays are run in WHITE plates.**



## HTRF<sup>®</sup> Terbium cryptate donor / Green acceptor readout Setup recommendations for SpectraMax<sup>®</sup> M5<sup>e</sup>

Two sequential measurements should be carried out: at 620 nm for the cryptate emission, and at 520 nm for the specific signal emitted by the acceptor. The ratio of the two fluorescence intensities 520/620 (acceptor/donor) enables the calculation of Delta F (%) which represents the relative energy transfer rate for each sample.

SpectraMax<sup>®</sup> M5e readers must be appropriately configured for HTRF<sup>®</sup> readout by setting up the measurement conditions in the SoftMax Pro software. In particular, these parameters should be entered as defined in the table below. No special upgrade is required for HTRF<sup>®</sup> readout, as it is a monochromator-based instrument.

<b>Read mode</b>	
	Time-Resolved Fluorescence (RFUs)
Integration delay	50 µs
Integration	300 µs
	Top read
<b>Wavelengths</b>	
Excitation 1	340 nm
Emission 1	520 nm
Cut off for emission 1	495 nm
Excitation 2	340 nm
Emission 2	620 nm
Cut off for emission 2	570 nm
<b>Sensitivity</b>	
Readings	50 to 100
PMT	Optimal
<b>Column Wavelength</b>	
	Column Priority

**This reader only allows high performance HTRF measurement when assays are run in WHITE plates.**



## HTRF® Terbium cryptate donor / Red acceptor readout Setup recommendations for SpectraMax® M5e

Two sequential measurements should be carried out: at 620 nm for the cryptate emission, and at 665 nm for the specific signal emitted by the acceptor. The ratio of the two fluorescence intensities 665/620 (acceptor/donor) enables the calculation of Delta F (%) which represents the relative energy transfer rate for each sample.

SpectraMax® M5e readers must be appropriately configured for HTRF® readout by setting up the measurement conditions in the SoftMax Pro software. In particular, these parameters should be entered as defined in the table below. No special upgrade is required for HTRF® readout, as it is a monochromator-based instrument.

<b>Read mode</b>	
	Time-Resolved Fluorescence (RFUs)
Integration delay	50 µs
Integration	300 µs
	Top read
<b>Wavelengths</b>	
Excitation 1	340 nm
Emission 1	665 nm
Cut off for emission 1	630 nm
Excitation 2	340 nm
Emission 2	620 nm
Cut off for emission 2	570 nm
<b>Sensitivity</b>	
Readings	50 to 100
PMT	Optimal
<b>Column Wavelength</b>	
	Column Priority

**This reader only allows high performance HTRF measurement when assays are run in WHITE plates.**

