

HTRF[®] Europium cryptate donor / Red acceptor readout Setup recommendations for Infinite[®] M1000

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.

Infinite[®] M1000 readers must be appropriately configured for HTRF[®] readout by setting up the measurement conditions in the "multilabeling" function of Tecan i-Control[™] software. In particular, these parameters should be entered as below. No special upgrade is required for HTRF[®] readout, as it is a monochromator-based instrument:

	Label 1	Label 2
Measurement	Fluorescence intensity	Fluorescence intensity
Excitation wavelength	317nm / 20nm	317nm / 20nm
Emission wavelength	665nm / 10nm	620nm / 10nm
Mode	Top	Top
Flashes	Mode 2 [100Hz] : 100	Mode 2 [100Hz] : 100
Gain	Optimal	Optimal
Z position	Must be calculated from the well giving the highest signal	Must be calculated from the well giving the highest signal
Lag time	60µs	60µs
Integration time	500µs	500µs

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



HTRF[®] Terbium cryptate donor / Green acceptor readout Setup recommendations for Infinite[®] M1000

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.

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	Label 1	Label 2
Measurement	Fluorescence intensity	Fluorescence intensity
Excitation wavelength	340nm / 20nm	340nm / 20nm
Emission wavelength	520nm / 10nm	620nm / 10nm
Mode	Top	Top
Flashes	Mode 2 [100Hz] : 100	Mode 2 [100Hz] : 100
Gain	Optimal	Optimal
Z position	Must be calculated from the well giving the highest signal	Must be calculated from the well giving the highest signal
Lag time	60µs	60µs
Integration time	500µs	500µs

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 Infinite[®] M1000

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Excitation wavelength	340nm / 20nm	340nm / 20nm
Emission wavelength	665nm / 10nm	620nm / 10nm
Mode	Top	Top
Flashes	Mode 2 [100Hz] : 100	Mode 2 [100Hz] : 100
Gain	Optimal	Optimal
Z position	Must be calculated from the well giving the highest signal	Must be calculated from the well giving the highest signal
Lag time	60µs	60µs
Integration time	500µs	500µs

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

